(51) International Patent Classification[7]: C07H 21/24,
C12N 15/00

(21) International Application Number:
PCT/US2003/020389

(22) International Filing Date: 26 June 2003 (26.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/392,415	26 June 2002 (26.06.2002)	US
60/441,377	21 January 2003 (21.01.2003)	US
60/441,502	21 January 2003 (21.01.2003)	US
60/441,405	21 January 2003 (21.01.2003)	US
60/441,447	21 January 2003 (21.01.2003)	US
60/441,392	21 January 2003 (21.01.2003)	US
60/441,381	21 January 2003 (21.01.2003)	US

(71) Applicants (for all designated States except US): TRANS-
GENRX, INC. [US/US]; Suite 300, 1755 Wittington Place
Drive, Dallas, TX 75234 (US). THE BOARD OF SUPER-
VISORS OF LOUISIANA STATE UNIVERSITY AND
AGRICULTURAL AND MECHANICAL COLLEGE
[US/US]; P.O. Box 25055, Baton Rouge, LA 70894-5055
(US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): COOPER, Richard,
K. [US/US]; 111 Pecan Meadow Drive, Baton Rouge,
LA 70810 (US). CADD, Gary, G. [US/US]; 501 Turner
Road, Apartment 1111, Grapevine, TX 76051 (US).
FIORETTI, William, C. [US/US]; 2225 Lakeridge Road
Drive, Grapevine, TX 76051 (US). DEBOER, Kenneth,
F. [US/US]; 10720 Gee Norman Road, Belgrade, MT
59714 (US).

(74) Agents: PRATT, John, S. et al.; Kilpatrick Stockton LLP,
Suite 2800, 1100 Peachtree Street, Atlanta, GA 30309
(US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
— as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations

Published:
— with international search report

(88) Date of publication of the international search report:
28 October 2004

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: GENE REGULATION IN TRANSGENIC ANIMALS USING A TRANSPOSON-BASED VECTOR

(57) Abstract: Administration of modified transposon-based vectors has been used to achieve stable incorporation of exogenous
genes into animals. These transgenic animals produce transgenic progeny. Further, these transgenic animals produce large quantities
of desired molecules encoded by the transgene. Transgenic egg-laying animals produce large quantities of desired molecules encoded
by the transgene and deposit these molecules in the egg.

5

GENE REGULATION IN TRANSGENIC ANIMALS USING A TRANSPOSON-BASED VECTOR

FIELD OF THE INVENTION

The present invention relates generally to cell-specific gene regulation in
transgenic animals. Animals may be made transgenic through administration of a
20   transposon-based vector through any method of administration including pronuclear
injection, or intraembryonic, intratesticular, intraoviductal or intravenous
administration. These transgenic animals contain the gene of interest in all cells,
including germ cells. Animals may also be made transgenic by targeting specific cells
for uptake and gene incorporation of the transposon-based vectors. Stable
25   incorporation of a gene of interest into cells of the transgenic animals is demonstrated
by expression of the gene of interest in a cell, wherein expression is regulated by a
promoter sequence. The promoter sequence may be provided as a transgene along
with the gene of interest or may be endogenous to the cell. The promoter sequence
may be constitutive or inducible, wherein inducible promoters include tissue-specific
30   promoters, developmentally regulated promoters and chemically inducible promoters.

BACKGROUND OF THE INVENTION

Transgenic animals are desirable for a variety of reasons, including their
potential as biological factories to produce desired molecules for pharmaceutical,
35   diagnostic and industrial uses. This potential is attractive to the industry due to the
inadequate capacity in facilities used for recombinant production of desired molecules
and the increasing demand by the pharmaceutical industry for use of these facilities.
Numerous attempts to produce transgenic animals have met several problems,

including low rates of gene incorporation and unstable gene incorporation. Accordingly, improved gene technologies are needed for the development of transgenic animals for the production of desired molecules.

5   Improved gene delivery technologies are also needed for the treatment of disease in animals and humans. Many diseases and conditions can be treated with gene-delivery technologies, which provide a gene of interest to a patient suffering from the disease or the condition. An example of such disease is Type 1 diabetes. Type 1 diabetes is an autoimmune disease that ultimately results in destruction of the insulin producing β-cells in the pancreas. Although patients with Type 1 diabetes

10   may be treated adequately with insulin injections or insulin pumps, these therapies are only partially effective. Insulin replacement, such as via insulin injection or pump administration, cannot fully reverse the defect in the vascular endothelium found in the hyperglycemic state (Pieper et al., 1996. Diabetes Res. Clin. Pract. Suppl. S157-S162). In addition, hyper- and hypoglycemia occurs frequently despite intensive

15   home blood glucose monitoring. Finally, careful dietary constraints are needed to maintain an adequate ratio of consumed calories consumed. This often causes major psychosocial stress for many diabetic patients. Development of gene therapies providing delivery of the insulin gene into the pancreas of diabetic patients could overcome many of these problems and result in improved life expectancy and quality

20   of life.

Several of the prior art gene delivery technologies employed viruses that are associated with potentially undesirable side effects and safety concerns. The majority of current gene-delivery technologies useful for gene therapy rely on virus-based delivery vectors, such as adeno and adeno-associated viruses, retroviruses, and other

25   viruses, which have been attenuated to no longer replicate. (Kay, M.A., et al. 2001. Nature Medicine 7:33-40).

There are multiple problems associated with the use or viral vectors. First, they are not tissue-specific. In fact, a gene therapy trial using adenovirus was recently halted because the vector was present in the patient's sperm (Gene trial to proceed

30   despite fears that therapy could change child's genetic makeup. The New York Times, December 23, 2001). Second, viral vectors are likely to be transiently incorporated, which necessitates re-treating a patient at specified time intervals. (Kay, M.A., et al. 2001. Nature Medicine 7:33-40). Third, there is a concern that a viral-based vector could revert to its virulent form and cause disease. Fourth, viral-based

35   vectors require a dividing cell for stable integration. Fifth, viral-based vectors indiscriminately integrate into various cells and tissues, which can result in undesirable germline integration. Sixth, the required high titers needed to achieve the desired effect have resulted in the death of one patient and they are believed to be

responsible for induction of cancer in a separate study. (Science, News of the Week, October 4, 2002).

Accordingly, what is needed is a new vector to produce transgenic animals and humans with stably incorporated genes, which vector does not cause disease or
5   other unwanted side effects. There is also a need for DNA constructs that would be stably incorporated into the tissues and cells of animals and humans, including cells in the resting state, which are not replicating. There is a further recognized need in the art for DNA constructs capable of delivering genes to specific tissues and cells of animals and humans.

10   When incorporating a gene of interest into an animal for the production of a desired protein or when incorporating a gene of interest in an animal or human for the treatment of a disease, it is often desirable to selectively activate incorporated genes using inducible promoters. These inducible promoters are regulated by substances either produced or recognized by the transcription control elements within the cell in
15   which the gene is incorporated. In many instances, control of gene expression is desired in transgenic animals or humans so that incorporated genes are selectively activated at desired times and/or under the influence of specific substances. Accordingly, what is needed is a means to selectively activate genes introduced into the genome of cells of a transgenic animal or human. This can be taken a step further
20   to cause incorporation to be tissue-specific, which prevents widespread gene incorporation throughout a patient's body (animal or human). This decreases the amount of DNA needed for a treatment, decreases the chance of incorporation in gametes, and targets gene delivery, incorporation, and expression to the desired tissue where the gene is needed to function.

25

SUMMARY OF THE INVENTION

The present invention addresses the problems described above by providing new, effective and efficient compositions for producing transgenic animals and for treating disease in animals or humans. Transgenic animals include all egg-laying
30   animals and milk-producing animals. Transgenic animals further include but are not limited to avians, fish, amphibians, reptiles, insects, mammals and humans. In a preferred embodiment, the animal is an avian animal. In another preferred embodiment, the animal is a milk-producing animal, including but not limited to bovine, porcine, ovine and equine animals. Animals are made transgenic through
35   administration of a composition comprising a transposon-based vector designed for stable incorporation of a gene of interest for production of a desired protein, together

ATLLIB02 133492.1

with an acceptable carrier. A transfection reagent is optionally added to the composition before administration.

The transposon-based vectors of the present invention include a transposase, operably-linked to a first promoter, and a coding sequence for a protein or peptide of 5 interest operably-linked to a second promoter, wherein the coding sequence for the protein or peptide of interest and its operably-linked promoter are flanked by transposase insertion sequences recognized by the transposase. The transposon-based vector also includes the following characteristics: a) one or more modified Kozak sequences comprising ACCATG (SEQ ID NO:13) at the 3' end of the first promoter 10 to enhance expression of the transposase; b) modifications of the codons for the first several N-terminal amino acids of the transposase, wherein the nucleotide at the third base position of each codon was changed to an A or a T without changing the corresponding amino acid; c) addition of one or more stop codons to enhance the termination of transposase synthesis; and/or, d) addition of an effective polyA 15 sequence operably-linked to the transposase to further enhance expression of the transposase gene.

Use of the compositions of the present invention results in highly efficient and stable incorporation of a gene of interest into the genome of transfected animals. For example, transgenic avians have been mated and produce transgenic progeny in the 20 G1 generation. The transgenic progeny have been mated and produce transgenic progeny in the G2 generation.

The present invention also provides for tissue-specific incorporation and/or expression of a gene of interest. Tissue-specific incorporation of a gene of interest may be achieved by placing the transposase gene under the control of a tissue-specific 25 promoter, whereas tissue-specific expression of a gene of interest may be achieved by placing the gene of interest under the control of a tissue-specific promoter. In some embodiments, the gene of interest is transcribed under the influence of an ovalbumin, or other oviduct specific, promoter. Linking the gene of interest to an oviduct specific promoter in an egg-laying animal results in synthesis of a desired molecule and 30 deposition of the desired molecule in a developing egg. The present invention further provides for stable incorporation and expression of genes in the epithelial cells of the mammary gland in milk-producing animals. Transcription of the gene of interest in the epithelial cells of the mammary gland results in synthesis of a desired molecule

4

and deposition of the desired molecule in the milk. A preferred molecule is a protein. In some embodiments, the desired molecule deposited in the milk is an antiviral protein, an antibody, or a serum protein.

In other embodiments, specific incorporation of the proinsulin gene into liver
5   cells of a diabetic animal results in the improvement of the animal's condition. Such improvement is achieved by placing a transposase gene under the control of a liver-specific promoter, which drives integration of the gene of interest in liver cells of the diabetic animal.

The present invention advantageously produces a high number of transgenic
10 animals having a gene of interest stably incorporated. These transgenic animals successfully pass the desired gene to their progeny. The transgenic animals of the present invention also produce large amounts of a desired molecule encoded by the transgene. Transgenic egg-laying animals, particularly avians, produce large amounts of a desired protein that is deposited in the egg for rapid harvest and purification.
15 Transgenic milk-producing animals produce large amounts of a desired protein that is deposited in the milk for rapid harvest and purification.

Any desired gene may be incorporated into the novel transposon-based vectors of the present invention in order to synthesize a desired molecule in the transgenic animals. Proteins, peptides and nucleic acids are preferred desired molecules to be
20 produced by the transgenic animals of the present invention. Particularly preferred proteins are antibody proteins.

This invention provides a composition useful for the production of transgenic hens capable of producing substantially high amounts of a desired protein or peptide. Entire flocks of transgenic birds may be developed very quickly in order to produce
25 industrial amounts of desired molecules. The present invention solves the problems inherent in the inadequate capacity of fermentation facilities used for bacterial production of molecules and provides a more efficient and economical way to produce desired molecules. Accordingly, the present invention provides a means to produce large amounts of therapeutic, diagnostic and reagent molecules.
30 Transgenic chickens are excellent in terms of convenience and efficiency of manufacturing molecules such as proteins and peptides. Starting with a single transgenic rooster, thousands of transgenic offspring can be produced within a year. (In principle, up to forty million offspring could be produced in just three

5

generations). Each transgenic female is expected to lay at least 250 eggs/year, each potentially containing hundreds of milligrams of the selected protein. Flocks of chickens numbering in the hundreds of thousands are readily handled through established commercial systems. The technologies for obtaining eggs and

5   fractionating them are also well known and widely accepted. Thus, for each therapeutic, diagnostic, or other protein of interest, large amounts of a substantially pure material can be produced at relatively low incremental cost.

A wide range of recombinant peptides and proteins can be produced in transgenic egg-laying animals and milk-producing animals. Enzymes, hormones,

10   antibodies, growth factors, serum proteins, commodity proteins, biological response modifiers, peptides and designed proteins may all be made through practice of the present invention. For example, rough estimates suggest that it is possible to produce in bulk growth hormone, insulin, or Factor VIII, and deposit them in transgenic egg whites, for an incremental cost in the order of one dollar per gram. At such prices it

15   is feasible to consider administering such medical agents by inhalation or even orally, instead of through injection. Even if bioavailability rates through these avenues were low, the cost of a much higher effective-dose would not be prohibitive.

In one embodiment, the egg-laying transgenic animal is an avian. The method of the present invention may be used in avians including Ratites, Psittaciformes,

20   Falconiformes, Piciformes, Strigiformes, Passeriformes, Coraciformes, Ralliformes, Cuculiformes, Columbiformes, Galliformes, Anseriformes, and Herodiones. Preferably, the egg-laying transgenic animal is a poultry bird. More preferably, the bird is a chicken, turkey, duck, goose or quail. Another preferred bird is a ratite, such as, an emu, an ostrich, a rhea, or a cassowary. Other preferred birds are partridge,

25   pheasant, kiwi, parrot, parakeet, macaw, falcon, eagle, hawk, pigeon, cockatoo, song birds, jay bird, blackbird, finch, warbler, canary, toucan, mynah, or sparrow.

In another embodiment, the transgenic animal is a milk-producing animal, including but not limited to bovine, ovine, porcine, equine, and primate animals. Milk-producing animals include but are not limited to cows, goats, horses, pigs,

30   buffalo, rabbits, non-human primates, and humans.

Accordingly, it is an object of the present invention to provide novel transposon-based vectors.

ATLLIB02 1334921

It is another object of the present invention to provide novel transposon-based vectors that encode for the production of desired proteins or peptides in cells.

It is an object of the present invention to produce transgenic animals through administration of a transposon-based vector.

5   Another object of the present invention is to produce transgenic animals through administration of a transposon-based vector, wherein the transgenic animals produce desired proteins or peptides.

Yet another object of the present invention is to produce transgenic animals through administration of a transposon-based vector, wherein the transgenic animals

10   produce desired proteins or peptides and deposit the proteins or peptides in eggs or milk.

It is a further object of the present invention to produce transgenic animals through intraembryonic, intratesticular or intraoviductal administration of a transposon-based vector.

15   It is further an object of the present invention to provide a method to produce transgenic animals through administration of a transposon-based vector that are capable of producing transgenic progeny.

Yet another object of the present invention is to provide a method to produce transgenic animals through administration of a transposon-based vector that are

20   capable of producing a desired molecule, such as a protein, peptide or nucleic acid.

Another object of the present invention is to provide a method to produce transgenic animals through administration of a transposon-based vector, wherein such administration results in modulation of endogenous gene expression.

It is another object of the present invention to provide transposon-vectors

25   useful for cell- or tissue-specific expression of a gene of interest in an animal or human with the purpose of gene therapy.

It is yet another object of the present invention to provide a method to produce transgenic avians through administration of a transposon-based vector that are capable of producing proteins, peptides or nucleic acids.

30   It is another object of the present invention to produce transgenic animals through administration of a transposon-based vector encoding an antibody or a fragment thereof.

ATL.LIB02 133492.1

Still another object of the present invention is to provide a method to produce transgenic avians through administration of a transposon-based vector that are capable of producing proteins or peptides and depositing these proteins or peptides in the egg.

Another object of the present invention is to provide transgenic avians that
5   contain a stably incorporated transgene.

Still another object of the present invention is to provide eggs containing desired proteins or peptides encoded by a transgene incorporated into the transgenic avian that produces the egg.

It is further an object of the present invention to provide a method to produce
10   transgenic milk-producing animals through administration of a transposon-based vector that are capable of producing proteins, peptides or nucleic acids.

Still another object of the present invention is to provide a method to produce transgenic milk-producing animals through administration of a transposon-based vector that are capable of producing proteins or peptides and depositing these proteins
15   or peptides in their milk.

Another object of the present invention is to provide transgenic milk-producing animals that contain a stably incorporated transgene.

Another object of the present invention is to provide transgenic milk-producing animals that are capable of producing proteins or peptides and depositing
20   these proteins or peptides in their milk.

Yet another object of the present invention is to provide milk containing desired molecules encoded by a transgene incorporated into the transgenic milk-producing animals that produce the milk.

Still another object of the present invention is to provide milk containing
25   desired proteins or peptides encoded by a transgene incorporated into the transgenic milk-producing animals that produce the milk.

A further object of the present invention to provide a method to produce transgenic sperm through administration of a transposon-based vector to an animal.

A further object of the present invention to provide transgenic sperm that
30   contain a stably incorporated transgene.

An advantage of the present invention is that transgenic animals are produced with higher efficiencies than observed in the prior art.

ATL.LIB02 1334921

Another advantage of the present invention is that these transgenic animals possess high copy numbers of the transgene.

Another advantage of the present invention is that the transgenic animals produce large amounts of desired molecules encoded by the transgene.

Still another advantage of the present invention is that desired molecules are produced by the transgenic animals much more efficiently and economically than prior art methods, thereby providing a means for large scale production of desired molecules, particularly proteins and peptides.

According to the invention there is also provided a vector comprising:

a) a modified transposase gene operably-linked to a first promoter; wherein the nucleic acid sequence 3' to the first promoter comprises the sequence as set forth in SEQ ID NO:13, wherein SEQ ID NO:13 contains the Kozak sequence and a start codon for the transposase, and wherein at least one of the first twenty codons for the transposase gene are modified from the wild-type sequence by changing a nucleotide at a third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon, and

b) one or more genes of interest operably-linked to one or more additional promoters; and wherein the one or more genes of interest and their operably-linked promoters are flanked by transposase insertion sequences recognized by the transposase encoded by the modified transposase gene.

According to the invention there is also provided a method of producing a transgenic animal comprising administering to the animal a vector according to the invention.

According to the invention there is also provided an egg produced by the transgenic avian animal according to the invention, wherein the egg contains one or more desired proteins encoded by the one or more genes of interest.

According to the invention there is also provided a transgenic sperm produced by the transgenic animal produced according to the invention.

According to the invention there is also provided a method for producing a desired protein comprising:

a) administering to the animal a vector according to the invention; and

b) isolating the desired protein produced in the animal.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. Any

9

discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts schematically a transposon-based vector containing a transposase operably linked to a first promoter and a gene of interest operably-linked to a second promoter, wherein the gene of interest and its operably-linked promoter are flanked by insertion sequences (IS) recognized by the transposase. "Pro" designates a promoter. In this and subsequent figures, the size of the actual nucleotide sequence is not necessarily proportionate to the box representing that sequence.

Figure 2 depicts schematically a transposon-based vector for targeting deposition of a polypeptide in an egg white wherein Ov pro is the ovalbumin promoter, Ov protein is the ovalbumin protein and PolyA is a polyadenylation sequence. The TAG sequence includes a spacer, the gp41 hairpin loop from HIV I and a protein cleavage site.

Figure 3 depicts schematically a transposon-based vector for targeting deposition of a polypeptide in an egg white wherein Ovo pro is the ovomucoid promoter and Ovo SS is the ovomucoid signal sequence. The TAG sequence includes a spacer, the gp41 hairpin loop from HIV I and a protein cleavage site.

Figure 4 depicts schematically a transposon-based vector for targeting deposition of a polypeptide in an egg yolk wherein Vit pro is the vitellogenin promoter and Vit targ is the vitellogenin targeting sequence.

5   Figure 5 depicts schematically a transposon-based vector for expression of antibody heavy and light chains. Prepro indicates a prepro sequence from cecropin and pro indicates a pro sequence from cecropin.

Figure 6 depicts schematically a transposon-based vector for expression of
10   antibody heavy and light chains. Ent indicates an enterokinase cleavage sequence.

Figure 7 depicts schematically egg white targeted expression of antibody heavy and light chains from one vector in either tail-to-tail (Figure 7A) or tail-to-head (Figure 7B) configuration. In the tail-to-tail configuration, the ovalbumin signal
15   sequence adjacent to the gene for the light chain contains on its 3' end an enterokinase cleavage site (not shown) to allow cleavage of the signal sequence from the light chain, and the ovalbumin signal sequence adjacent to the gene for the heavy chain contains on its 5' end an enterokinase cleavage site (not shown) to allow cleavage of the signal sequence from the heavy chain. In the tail-to-head configuration, the
20   ovalbumin signal sequence adjacent to the gene for the heavy chain and the light chain contains on its 3' end an enterokinase cleavage site (not shown) to allow cleavage of the signal sequence from the heavy or light chain.

DETAILED DESCRIPTION OF THE INVENTION
25   The present invention provides a new, effective and efficient method of producing transgenic animals, particularly egg-laying animals and milk-producing animals, through administration of a composition comprising a transposon-based vector designed for stable incorporation of a gene of interest for production of a desired molecule.

30   Definitions
It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized.

10

The term "antibody" is used interchangeably with the term "immunoglobulin" and is defined herein as a protein synthesized by an animal or a cell of the immune system in response to the presence of a foreign substance commonly referred to as an "antigen" or an "immunogen". The term antibody includes fragments of antibodies.

5   Antibodies are characterized by specific affinity to a site on the antigen, wherein the site is referred to an "antigenic determinant" or an "epitope". Antigens can be naturally occurring or artificially engineered. Artificially engineered antigens include but are not limited to small molecules, such as small peptides, attached to haptens such as macromolecules, for example proteins, nucleic acids, or polysaccharides.

10   Artificially designed or engineered variants of naturally occurring antibodies and artificially designed or engineered antibodies not occurring in nature are all included in the current definition. Such variants include conservatively substituted amino acids and other forms of substitution as described in the section concerning proteins and polypeptides.

15   As used herein, the term "egg-laying animal" includes all amniotes such as birds, turtles, lizards and monotremes. Monotremes are egg-laying mammals and include the platypus and echidna. The term "bird" or "fowl," as used herein, is defined as a member of the Aves class of animals which are characterized as warm-blooded, egg-laying vertebrates primarily adapted for flying. Avians include, without

20   limitation, Ratites, Psittaciformes, Falconiformes, Piciformes, Strigiformes, Passeriformes, Coraciformes, Ralliformes, Cuculiformes, Columbiformes, Galliformes, Anseriformes, and Herodiones. The term "Ratite," as used herein, is defined as a group of flightless, mostly large, running birds comprising several orders and including the emus, ostriches, kiwis, and cassowaries. The term "Psittaciformes",

25   as used herein, includes parrots and refers to a monofamilial order of birds that exhibit zygodactylism and have a strong hooked bill. A "parrot" is defined as any member of the avian family Psittacidae (the single family of the Psittaciformes), distinguished by the short, stout, strongly hooked beak. The term "chicken" as used herein denotes chickens used for table egg production, such as egg-type chickens, chickens reared for

30   public meat consumption, or broilers, and chickens reared for both egg and meat production ("dual-purpose" chickens). The term "chicken" also denotes chickens produced by primary breeder companies, or chickens that are the parents,

11

grandparents, great-grandparents, etc. of those chickens reared for public table egg, meat, or table egg and meat consumption.

The term "egg" is defined herein as a large female sex cell enclosed in a porous, calcarous or leathery shell, produced by birds and reptiles. The term "ovum"

5 is defined as a female gamete, and is also known as an egg. Therefore, egg production in all animals other than birds and reptiles, as used herein, is defined as the production and discharge of an ovum from an ovary, or "ovulation". Accordingly, it is to be understood that the term "egg" as used herein is defined as a large female sex cell enclosed in a porous, calcarous or leathery shell, when a bird or reptile produces

10 it, or it is an ovum when it is produced by all other animals.

The term "milk-producing animal" refers herein to mammals including, but not limited to, bovine, ovine, porcine, equine, and primate animals. Milk-producing animals include but are not limited to cows, llamas, camels, goats, reindeer, zebu, water buffalo, yak, horses, pigs, rabbits, non-human primates, and humans.

15 The term "gene" is defined herein to include a coding region for a protein, peptide or polypeptide.

The term "vector" is used interchangeably with the terms "construct", "DNA construct" and "genetic construct" to denote synthetic nucleotide sequences used for manipulation of genetic material, including but not limited to cloning, subcloning,

20 sequencing, or introduction of exogenous genetic material into cells, tissues or organisms, such as birds. It is understood by one skilled in the art that vectors may contain synthetic DNA sequences, naturally occurring DNA sequences, or both. The vectors of the present invention are transposon-based vectors as described herein.

When referring to two nucleotide sequences, one being a regulatory sequence,

25 the term "operably-linked" is defined herein to mean that the two sequences are associated in a manner that allows the regulatory sequence to affect expression of the other nucleotide sequence. It is not required that the operably-linked sequences be directly adjacent to one another with no intervening sequence(s).

The term "regulatory sequence" is defined herein as including promoters,

30 enhancers and other expression control elements such as polyadenylation sequences, matrix attachment sites, insulator regions for expression of multiple genes on a single construct, ribosome entry/attachment sites, introns that are able to enhance expression, and silencers.

12

Transposon-Based Vectors

    While not wanting to be bound by the following statement, it is believed that the nature of the DNA construct is an important factor in successfully producing transgenic animals. The "standard" types of plasmid and viral vectors that have

5   previously been almost universally used for transgenic work in all species, especially avians, have low efficiencies and may constitute a major reason for the low rates of transformation previously observed. The DNA (or RNA) constructs previously used often do not integrate into the host DNA, or integrate only at low frequencies. Other factors may have also played a part, such as poor entry of the vector into target cells.

10   The present invention provides transposon-based vectors that can be administered to an animal that overcome the prior art problems relating to low transgene integration frequencies. Two preferred transposon-based vectors of the present invention in which a tranposase, gene of interest and other polynucleotide sequences may be introduced are termed pTnMCS (SEQ ID NO:36) and pTnMod (SEQ ID NO:1).

15   The transposon-based vectors of the present invention produce integration frequencies an order of magnitude greater than has been achieved with previous vectors. More specifically, intratesticular injections performed with a prior art transposon-based vector (described in U.S. Patent No. 5,719,055) resulted in 41% sperm positive roosters whereas intratesticular injections performed with the novel

20   transposon-based vectors of the present invention resulted in 77% sperm positive roosters. Actual frequencies of integration were estimated by either or both comparative strength of the PCR signal from the sperm and histological evaluation of the testes and sperm by quantitative PCR.

    The transposon-based vectors of the present invention include a transposase

25   gene operably-linked to a first promoter, and a coding sequence for a desired protein or peptide operably-linked to a second promoter, wherein the coding sequence for the desired protein or peptide and its operably-linked promoter are flanked by transposase insertion sequences recognized by the transposase. The transposon-based vector also includes one or more of the following characteristics: a) one or more modified Kozak

30   sequences comprising ACCATG (SEQ ID NO:13) at the 3' end of the first promoter to enhance expression of the transposase; b) modifications of the codons for the first several N-terminal amino acids of the transposase, wherein the third base of each codon was changed to an A or a T without changing the corresponding amino acid; c)

13

addition of one or more stop codons to enhance the termination of transposase synthesis; and, d) addition of an effective polyA sequence operably-linked to the transposase to further enhance expression of the transposase gene. Figure 1 shows a schematic representation of several components of the transposon-based vector. The

5   present invention further includes vectors containing more than one gene of interest, wherein a second or subsequent gene of interest is operably-linked to the second promoter or to a different promoter. It is also to be understood that the transposon-based vectors shown in the Figures are representational of the present invention and that the order of the vector elements may be different than that shown in the Figures,

10   that the elements may be present in various orientations, and that the vectors may contain additional elements not shown in the Figures.

Transposases and Insertion Sequences

In a further embodiment of the present invention, the transposase found in the transposase-based vector is an altered target site (ATS) transposase and the insertion

15   sequences are those recognized by the ATS transposase. However, the transposase located in the transposase-based vectors is not limited to a modified ATS transposase and can be derived from any transposase. Transposases known in the prior art include those found in AC7, Tn5SEQ1, Tn916, Tn951, Tn1721, Tn 2410, Tn1681, Tn1, Tn2, Tn3, Tn4, Tn5, Tn6, Tn9, Tn10, Tn30, Tn101, Tn903, Tn501, Tn1000 (γδ), Tn1681,

20   Tn2901, AC transposons, Mp transposons, Spm transposons, En transposons, Dotted transposons, Mu transposons, Ds transposons, dSpm transposons and I transposons. According to the present invention, these transposases and their regulatory sequences are modified for improved functioning as follows: a) the addition one or more modified Kozak sequences comprising ACCATG (SEQ ID NO:13) at the 3' end of

25   the promoter operably-linked to the transposase; b) a change of the codons for the first several amino acids of the transposase, wherein the third base of each codon was changed to an A or a T without changing the corresponding amino acid; c) the addition of one or more stop codons to enhance the termination of transposase synthesis; and/or, d) the addition of an effective polyA sequence operably-linked to

30   the transposase to further enhance expression of the transposase gene.

Although not wanting to be bound by the following statement, it is believed that the modifications of the first several N-terminal codons of the transposase gene increase transcription of the transposase gene, in part, by increasing strand

14

ATLLIB02 133492.1

dissociation. It is preferable that between approximately 1 and 20, more preferably 3 and 15, and most preferably between 4 and 12 of the first N-terminal codons of the transposase are modified such that the third base of each codon is changed to an A or a T without changing the encoded amino acid. In one embodiment, the first ten N-
5   terminal codons of the transposase gene are modified in this manner. It is also preferred that the transposase contain mutations that make it less specific for preferred insertion sites and thus increases the rate of transgene insertion as discussed in U.S. Patent No. 5,719,055.

In some embodiments, the transposon-based vectors are optimized for
10 expression in a particular host by changing the methylation patterns of the vector DNA. For example, prokaryotic methylation may be reduced by using a methylation deficient organism for production of the transposon-based vector. The transposon-based vectors may also be methylated to resemble eukaryotic DNA for expression in a eukaryotic host.

15 Transposases and insertion sequences from other analogous eukaryotic transposon-based vectors that can also be modified and used are, for example, the Drosophila P element derived vectors disclosed in U.S. Patent No. 6,291,243; the Drosophila mariner element described in Sherman et al. (1998); or the sleeping beauty transposon. See also Hackett et al. (1999); D. Lampe et al., 1999. Proc. Natl. Acad.
20 Sci. USA, 96:11428-11433; S. Fischer et al., 2001. Proc. Natl. Acad. Sci. USA, 98:6759-6764; L. Zagoraiou et al., 2001. Proc. Natl. Acad. Sci. USA, 98:11474-11478; and D. Berg et al. (Eds.), Mobile DNA, Amer. Soc. Microbiol. (Washington, D.C., 1989). However, it should be noted that bacterial transposon-based elements are preferred, as there is less likelihood that a eukaryotic transposase in the recipient
25 species will recognize prokaryotic insertion sequences bracketing the transgene.

Many transposases recognize different insertion sequences, and therefore, it is to be understood that a transposase-based vector will contain insertion sequences recognized by the particular transposase also found in the transposase-based vector. In a preferred embodiment of the invention, the insertion sequences have been
30 shortened to about 70 base pairs in length as compared to those found in wild-type transposons that typically contain insertion sequences of well over 100 base pairs.

While the examples provided below incorporate a "cut and insert" Tn10 based vector that is destroyed following the insertion event, the present invention also

encompasses the use of a "rolling replication" type transposon-based vector. Use of a rolling replication type transposon allows multiple copies of the transposon/transgene to be made from a single transgene construct and the copies inserted. This type of transposon-based system thereby provides for insertion of multiple copies of a

5   transgene into a single genome. A rolling replication type transposon-based vector may be preferred when the promoter operably-linked to gene of interest is endogenous to the host cell and present in a high copy number or highly expressed. However, use of a rolling replication system may require tight control to limit the insertion events to non-lethal levels. Tn1, Tn2, Tn3, Tn4, Tn5, Tn9, Tn21, Tn501, Tn551, Tn951,

10   Tn1721, Tn2410 and Tn2603 are examples of a rolling replication type transposon, although Tn5 could be both a rolling replication and a cut and insert type transposon.

        Stop Codons and PolyA Sequences

        In one embodiment, the transposon-based vector contains two stop codons operably-linked to the transposase and/or to the gene of interest. In an alternate

15   embodiment, one stop codon of UAA or UGA is operably linked to the transposase and/or to the gene of interest. As used herein an "effective polyA sequence" refers to either a synthetic or non-synthetic sequence that contains multiple and sequential nucleotides containing an adenine base (an A polynucleotide string) and that increases expression of the gene to which it is operably-linked. A polyA sequence may be

20   operably-linked to any gene in the transposon-based vector including, but not limited to, a transposase gene and a gene of interest. In one embodiment, a polyA sequence comprises the polynucleotide sequence provided in SEQ ID NO:28. A preferred polyA sequence is optimized for use in the host animal or human. In one embodiment, the polyA sequence is optimized for use in a bird, and more specifically,

25   a chicken. The chicken optimized polyA sequence generally contains a minimum of 60 base pairs, and more preferably between approximately 60 and several hundred base pairs, that precede the A polynucleotide string and thereby separate the stop codon from the A polynucleotide string. A chicken optimized polyA sequence may also have a reduced amount of CT repeats as compared to a synthetic polyA sequence.

30   In one embodiment of the present invention, the polyA sequence comprises a conalbumin polyA sequence as provided in SEQ ID NO:33 and as taken from GenBank accession # Y00407, base pairs 10651-11058.

<u>Promoters and Enhancers</u>

The first promoter operably-linked to the transposase gene and the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter. Constitutive promoters include, but are not limited to, immediate early cytomegalovirus (CMV) promoter, herpes simplex virus 1 (HSV1) immediate early promoter, SV40 promoter, lysozyme promoter, early and late CMV promoters, early and late HSV promoters, β-actin promoter, tubulin promoter, Rous-Sarcoma virus (RSV) promoter, and heat-shock protein (HSP) promoter. Inducible promoters include tissue-specific promoters, developmentally-regulated promoters and chemically inducible promoters. Examples of tissue-specific promoters include the glucose 6 phosphate (G6P) promoter, vitellogenin promoter, ovalbumin promoter, ovomucoid promoter, conalbumin promoter, ovotransferrin promoter, prolactin promoter, kidney uromodulin promoter, and placental lactogen promoter. In one embodiment, the vitellogenin promoter includes a polynucleotide sequence of SEQ ID NO:17. The G6P promoter sequence may be deduced from a rat G6P gene untranslated upstream region provided in GenBank accession number U57552.1. Examples of developmentally-regulated promoters include the homeobox promoters and several hormone induced promoters. Examples of chemically inducible promoters include reproductive hormone induced promoters and antibiotic inducible promoters such as the tetracycline inducible promoter and the zinc-inducible metallothionine promoter.

Other inducible promoter systems include the Lac operator repressor system inducible by IPTG (isopropyl beta-D-thiogalactoside) (Cronin, A. et al. 2001. Genes and Development, v. 15), ecdysone-based inducible systems (Hoppe, U. C. et al. 2000. Mol. Ther. 1:159-164); estrogen-based inducible systems (Braselmann, S. et al. 1993. Proc. Natl. Acad. Sci. 90:1657-1661); progesterone-based inducible systems using a chimeric regulator, GLVP, which is a hybrid protein consisting of the GAL4 binding domain and the herpes simplex virus transcriptional activation domain, VP16, and a truncated form of the human progesterone receptor that retains the ability to bind ligand and can be turned on by RU486 (Wang, et al. 1994. Proc. Natl. Acad. Sci. 91:8180-8184); CID-based inducible systems using chemical inducers of dimerization (CIDs) to regulate gene expression, such as a system wherein rapamycin induces dimerization of the cellular proteins FKBP12 and FRAP (Belshaw, P. J. et al. 1996. J.

17

Chem. Biol. 3:731-738; Fan, L. et al. 1999. Hum. Gene Ther. 10:2273-2285; Shariat, S.F. et al. 2001. Cancer Res. 61:2562-2571; Spencer, D.M. 1996. Curr. Biol. 6:839-847). Chemical substances that activate the chemically inducible promoters can be administered to the animal containing the transgene of interest via any method known

5   to those of skill in the art.

Other examples of cell or tissue-specific and constitutive promoters include but are not limited to smooth-muscle SM22 promoter, including chimeric SM22alpha/telokin promoters (Hoggatt A.M. et al., 2002. Circ Res. 91(12):1151-9); ubiquitin C promoter (Biochim Biophys Acta, 2003. Jan. 3;1625(1):52-63); Hsf2

10   promoter; murine COMP (cartilage oligomeric matrix protein) promoter; early B cell-specific mb-1 promoter (Sigvardsson M., et al., 2002. Mol. Cell Biol. 22(24):8539-51); prostate specific antigen (PSA) promoter (Yoshimura I. et al., 2002, J. Urol. 168(6):2659-64); exorh promoter and pineal expression-promoting element (Asaoka Y., et al., 2002. Proc. Natl. Acad. Sci. 99(24):15456-61); neural and liver ceramidase

15   gene promoters (Okino N. et al., 2002. Biochem. Biophys. Res. Commun. 299(1):160-6); PSP94 gene promoter/enhancer (Gabril M.Y. et al., 2002. Gene Ther. 9(23):1589-99); promoter of the human FAT/CD36 gene (Kuriki C., et al., 2002. Biol. Pharm. Bull. 25(11):1476-8); VL30 promoter (Staplin W.R. et al., 2002. Blood October 24, 2002); IL-10 promoter (Brenner S., et al., 2002. J. Biol. Chem. December

20   18, 2002).

Examples of avian promoters include, but are not limited to, promoters controlling expression of egg white proteins, such as ovalbumin, ovotransferrin (conalbumin), ovomucoid, lysozyme, ovomucin, g2 ovoglobulin, g3 ovoglobulin, ovoflavoprotein, ovostatin (ovomacroglobin), cystatin, avidin, thiamine-binding

25   protein, glutamyl aminopeptidase minor glycoprotein 1, minor glycoprotein 2; and promoters controlling expression of egg-yolk proteins, such as vitellogenin, very low-density lipoproteins, low density lipoprotein, cobalamin-binding protein, riboflavin-binding protein, biotin-binding protein (Awade, 1996. Z. Lebensm. Unters. Forsch. 202:1-14). An advantage of using the vitellogenin promoter is that it is active during

30   the egg-laying stage of an animal's life-cycle, which allows for the production of the protein of interest to be temporally connected to the import of the protein of interest into the egg yolk when the protein of interest is equipped with an appropriate targeting sequence.

Liver-specific promoters of the present invention include, but are not limited to, the following promoters, vitellogenin promoter, G6P promoter, cholesterol-7-alpha-hydroxylase (CYP7A) promoter, phenylalanine hydroxylase (PAH) promoter, protein C gene promoter, insulin-like growth factor I (IGF-I) promoter, bilirubin

5   UDP-glucuronosyltransferase promoter, aldolase B promoter, furin promoter, metallothioneine promoter, albumin promoter, and insulin promoter.

Also included in the present invention are promoters that can be used to target expression of a protein of interest into the milk of a milk-producing animal including, but not limited to, β lactoglobin promoter, whey acidic protein promoter, lactalbumin

10   promoter and casein promoter.

Promoters associated with cells of the immune system may also be used. Acute phase promoters such as interleukin (IL)-1 and IL-2 may be employed. Promoters for heavy and light chain Ig may also be employed. The promoters of the T cell receptor components CD4 and CD8, B cell promoters and the promoters of

15   CR2 (complement receptor type 2) may also be employed. Immune system promoters are preferably used when the desired protein is an antibody protein.

Also included in this invention are modified promoters/enhancers wherein elements of a single promoter are duplicated, modified, or otherwise changed. In one embodiment, steroid hormone-binding domains of the ovalbumin promoter are moved

20   from about -6.5 kb to within approximately the first 1000 base pairs of the gene of interest. Modifying an existing promoter with promoter/enhancer elements not found naturally in the promoter, as well as building an entirely synthetic promoter, or drawing promoter/enhancer elements from various genes together on a non-natural backbone, are all encompassed by the current invention.

25   Accordingly, it is to be understood that the promoters contained within the transposon-based vectors of the present invention may be entire promoter sequences or fragments of promoter sequences. For example, in one embodiment, the promoter operably linked to a gene of interest is an approximately 900 base pair fragment of a chicken ovalbumin promoter (SEQ ID NO:40). The constitutive and inducible

30   promoters contained within the transposon-based vectors may also be modified by the addition of one or more modified Kozak sequences of ACCATG (SEQ ID NO:13).

As indicated above, the present invention includes transposon-based vectors containing one or more enhancers. These enhancers may or may not be operably-

19

linked to their native promoter and may be located at any distance from their operably-linked promoter. A promoter operably-linked to an enhancer is referred to herein as an "enhanced promoter." The enhancers contained within the transposon-based vectors are preferably enhancers found in birds, and more preferably, an

5   ovalbumin enhancer, but are not limited to these types of enhancers. In one embodiment, an approximately 675 base pair enhancer element of an ovalbumin promoter is cloned upstream of an ovalbumin promoter with 300 base pairs of spacer DNA separating the enhancer and promoter. In one embodiment, the enhancer used as a part of the present invention comprises base pairs 1-675 of a Chicken Ovalbumin

10   enhancer from GenBank accession #S82527.1. The polynucleotide sequence of this enhancer is provided in SEQ ID NO:37.

       Also included in some of the transposon-based vectors of the present invention are cap sites and fragments of cap sites. In one embodiment, approximately 50 base pairs of a 5' untranslated region wherein the capsite resides are added on the 3' end of

15   an enhanced promoter or promoter. An exemplary 5' untranslated region is provided in SEQ ID NO:38. A putative cap-site residing in this 5' untranslated region preferably comprises the polynucleotide sequence provided in SEQ ID NO:39.

       In one embodiment of the present invention, the first promoter operably-linked to the transposase gene is a constitutive promoter and the second promoter operably-

20   linked to the gene of interest is a tissue-specific promoter. In this embodiment, use of the first constitutive promoter allows for constitutive activation of the transposase gene and incorporation of the gene of interest into virtually all cell types, including the germline of the recipient animal. Although the gene of interest is incorporated into the germline generally, the gene of interest is only expressed in a tissue-specific

25   manner. It should be noted that cell- or tissue-specific expression as described herein does not require a complete absence of expression in cells or tissues other than the preferred cell or tissue. Instead, "cell-specific" or "tissue-specific" expression refers to a majority of the expression of a particular gene of interest in the preferred cell or tissue, respectively.

30   When incorporation of the gene of interest into the germline is not preferred, the first promoter operably-linked to the transposase gene can be a tissue-specific promoter. For example, transfection of a transposon-based vector containing a transposase gene operably-linked to a liver-specific promoter such as the G6P

promoter or vitellogenin promoter provides for activation of the transposase gene and incorporation of the gene of interest in the cells of the liver but not into the germline and other cells generally. In this second embodiment, the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter.

5   In a preferred embodiment, both the first promoter and the second promoter are a G6P promoter. In embodiments wherein tissue-specific expression or incorporation is desired, it is preferred that the transposon-based vector is administered directly to the tissue of interest or to an artery leading to the tissue of interest.

Accordingly, cell specific promoters may be used to enhance transcription in

10   selected tissues. In birds, for example, promoters that are found in cells of the fallopian tube, such as ovalbumin, conalbumin, ovomucoid and/or lysozyme, are used in the vectors to ensure transcription of the gene of interest in the epithelial cells and tubular gland cells of the fallopian tube, leading to synthesis of the desired protein encoded by the gene and deposition into the egg white. In mammals, promoters

15   specific for the epithelial cells of the alveoli of the mammary gland, such as prolactin, insulin, beta lactoglobin, whey acidic protein, lactalbumin, casein, and/or placental lactogen, are used in the design of vectors used for transfection of these cells for the production of desired proteins for deposition into the milk. In liver cells, the G6P promoter may be employed to drive transcription of the gene of interest for protein

20   production. Proteins made in the liver of birds may be delivered to the egg yolk.

In order to achieve higher or more efficient expression of the transposase gene, the promoter and other regulatory sequences operably-linked to the transposase gene may be those derived from the host. These host specific regulatory sequences can be tissue specific as described above or can be of a constitutive nature. For

25   example, an avian actin promoter and its associated polyA sequence can be operably-linked to a transposase in a transposase-based vector for transfection into an avian. Examples of other host specific promoters that could be operably-linked to the transposase include the myosin and DNA or RNA polymerase promoters.

Directing Sequences

30   In some embodiments of the present invention, the gene of interest is operably-linked to a directing sequence or a sequence that provides proper conformation to the desired protein encoded by the gene of interest. As used herein, the term "directing sequence" refers to both signal sequences and targeting sequences.

21

An egg directing sequence includes, but is not limited to, an ovomucoid signal sequence, an ovalbumin signal sequence and a vitellogenin targeting sequence. The term "signal sequence" refers to an amino acid sequence, or the polynucleotide sequence that encodes the amino acid sequence, that directs the protein to which it is

5   linked to the endoplasmic reticulum in a eukaryote, and more preferably the translocational pores in the endoplasmic reticulum, or the plasma membrane in a prokaryote, or mitochondria, such us for the purpose of gene therapy of mitochondrial diseases. Signal and targeting sequences can be used to direct a desired protein into, for example, the milk, when the transposon-based vectors are administered to a milk-

10   producing animal.

Signal sequences can also be used to direct a desired protein into, for example, a secretory pathway for incorporation into the egg yolk or the egg white, when the transposon-based vectors are administered to a bird or other egg-laying animal. One example of such a transposon-based vector is provided in Figure 3 wherein the gene

15   of interest is operably linked to the ovomucoid signal sequence. The present invention also includes a gene of interest operably-linked to a second gene containing a signal sequence. An example of such an embodiment is shown in Figure 2 wherein the gene of interest is operably-linked to the ovalbumin gene that contains an ovalbumin signal sequence. Other signal sequences that can be included in the

20   transposon-based vectors include, but are not limited to the ovotransferrin and lysozyme signal sequences.

As also used herein, the term "targeting sequence" refers to an amino acid sequence, or the polynucleotide sequence encoding the amino acid sequence, which amino acid sequence is recognized by a receptor located on the exterior of a cell.

25   Binding of the receptor to the targeting sequence results in uptake of the protein or peptide operably-linked to the targeting sequence by the cell. One example of a targeting sequence is a vitellogenin targeting sequence that is recognized by a vitellogenin receptor (or the low density lipoprotein receptor) on the exterior of an oocyte. In one embodiment, the vitellogenin targeting sequence includes the

30   polynucleotide sequence of SEQ ID NO:18. In another embodiment, the vitellogenin targeting sequence includes all or part of the vitellogenin gene. Other targeting sequences include VLDL and Apo E, which are also capable of binding the vitellogenin receptor. Since the ApoE protein is not endogenously expressed in birds,

its presence may be used advantageously to identify birds carrying the transposon-based vectors of the present invention.

<u>Genes of Interest Encoding Desired Proteins</u>

A gene of interest selected for stable incorporation is designed to encode any desired protein or peptide or to regulate any cellular response. In some embodiments, the desired proteins or peptides are deposited in an egg or in milk. It is to be understood that the present invention encompasses transposon-based vectors containing multiple genes of interest. The multiple genes of interest may each be operably-linked to a separate promoter and other regulatory sequence(s) or may all be operably-linked to the same promoter and other regulatory sequences(s). In one embodiment, multiple gene of interest are linked to a single promoter and other regulatory sequence(s) and each gene of interest is separated by a cleavage site or a pro portion of a signal sequence.

Protein and peptide hormones are a preferred class of proteins in the present invention. Such protein and peptide hormones are synthesized throughout the endocrine system and include, but are not limited to, hypothalamic hormones and hypophysiotropic hormones, anterior, intermediate and posterior pituitary hormones, pancreatic islet hormones, hormones made in the gastrointestinal system, renal hormones, thymic hormones, parathyroid hormones, adrenal cortical and medullary hormones. Specifically, hormones that can be produced using the present invention include, but are not limited to, chorionic gonadotropin, corticotropin, erythropoietin, glucagons, IGF-1, oxytocin, platelet-derived growth factor, calcitonin, follicle-stimulating hormone, leutinizing hormone, thyroid-stimulating hormone, insulin, gonadotropin-releasing hormone and its analogs, vasopressin, octreotide, somatostatin, prolactin, adrenocorticotropic hormone, antidiuretic hormone, thyrotropin-releasing hormone (TRH), growth hormone-releasing hormone (GHRH), dopamine, melatonin, thyroxin (T_4), parathyroid hormone (PTH), glucocorticoids such as cortisol, mineralocorticoids such as aldosterone, androgens such as testosterone, adrenaline (epinephrine), noradrenaline (norepinephrine), estrogens such as estradiol, progesterone, glucagons, calcitrol, calciferol, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin (CCK), neuropeptide Y, ghrelin, PYY_{3-36}, angiotensinogen, thrombopoietin, and leptin. By using appropriate polynucleotide sequences, species-specific hormones may be made by transgenic animals.

23

In one embodiment of the present invention, the gene of interest is a proinsulin gene and the desired molecule is insulin. Proinsulin consists of three parts: a C-peptide and two long strands of amino acids (called the alpha and beta chains) that later become linked together to form the insulin molecule. Figures 2 and 3 are

5   schematics of transposon-based vector constructs containing a proinsulin gene operably-linked to an ovalbumin promoter and ovalbumin protein or an ovomucoid promoter and ovomucoid signal sequence, respectively. In these embodiments, proinsulin is expressed in the oviduct tubular gland cells and then deposited in the egg white. One example of a proinsulin polynucleotide sequence is shown in SEQ ID

10   NO:21, wherein the C-peptide cleavage site spans from Arg at position 31 to Arg at position 65.

Serum proteins including lipoproteins such as high density lipoprotein (HDL), HDL-Milano and low density lipoprotein, albumin, clotting cascade factors, factor VIII, factor IX, fibrinogen, and globulins are also included in the group of desired

15   proteins of the present invention. Immunoglobulins are one class of desired globulin molecules and include but are not limited to IgG, IgM, IgA, IgD, IgE, IgY, lambda chains, kappa chains and fragments thereof; Fc fragments, and Fab fragments. Desired antibodies include, but are not limited to, naturally occurring antibodies, human antibodies, humanized antibodies, and hybrid antibodies. Genes encoding

20   modified versions of naturally occurring antibodies or fragments thereof and genes encoding artificially designed antibodies or fragments thereof may be incorporated into the transposon-based vectors of the present invention. Desired antibodies also include antibodies with the ability to bind specific ligands, for example, antibodies against proteins associated with cancer-related molecules, such as anti-her 2, or anti-

25   CA125. Accordingly, the present invention encompasses a transposon-based vector containing one or more genes encoding a heavy immunoglobulin (Ig) chain and a light Ig chain. Further, more than one gene encoding for more than one antibody may be administered in one or more transposon-based vectors of the present invention. In this manner, an egg may contain more than one type of antibody in the egg white, the

30   egg yolk or both.

In one embodiment, a transposon-based vector contains a heavy Ig chain and a light Ig chain, both operably linked to a promoter. Figures 5 and 6 schematically depict exemplary constructs of this embodiment. More specifically, Figure 5 shows a

24

construct containing a cecropin pre-pro sequence and a cecropin pro sequence, wherein the pre sequence functions to direct the resultant protein into the endoplasmic reticulum and the pro sequences and the pro sequences are cleaved upon secretion of the protein from a cell into which the construct has been transfected. Figure 6 shows

5   a construct containing an enterokinase cleavage site. In this embodiment, it may be required to further remove several additional amino acids from the light chain following cleavage by enterokinase. In another embodiment, the transposon-based vector comprises a heavy Ig chain operably-linked to one promoter and a light Ig chain operably-linked to another promoter. Figure 7 schematically depicts an

10   exemplary construct of this embodiment. The present invention also encompasses a transposon-based vector containing genes encoding portions of a heavy Ig chain and/or portions of a light Ig chain. The present invention further includes a transposon-based vector containing a gene that encodes a fusion protein comprising a heavy and/or light Ig chain, or portions thereof.

15   Antibodies used as therapeutic reagents include but are not limited to antibodies for use in cancer immunotherapy against specific antigens, or for providing passive immunity to an animal or a human against an infectious disease or a toxic agent. Antibodies used as diagnostic reagents include, but are not limited to antibodies that may be labeled and detected with a detector, for example antibodies

20   with a fluorescent label attached that may be detected following exposure to specific wavelengths. Such labeled antibodies may be primary antibodies directed to a specific antigen, for example, rhodamine-labeled rabbit anti-growth hormone, or may be labeled secondary antibodies, such as fluorescein-labeled goat-anti chicken IgG. Such labeled antibodies are known to one of ordinary skill in the art. Labels useful

25   for attachment to antibodies are also known to one of ordinary skill in the art. Some of these labels are described in the "Handbook of Fluorescent Probes and Research Products", ninth edition, Richard P. Haugland (ed) Molecular Probes, Inc. Eugene, OR), which is incorporated herein in its entirety.

          Antibodies produced with using the present invention may be used as

30   laboratory reagents for numerous applications including radioimmunoassay, western blots, dot blots, ELISA, immunoaffinity columns and other procedures requiring antibodies as known to one of ordinary skill in the art. Such antibodies include

25

ATL.LIB02 1334921

primary antibodies, secondary antibodies and tertiary antibodies, which may be labeled or unlabeled.

Antibodies that may be made with the practice of the present invention include, but are not limited to primary antibodies, secondary antibodies, designer
5   antibodies, anti-protein antibodies, anti-peptide antibodies, anti-DNA antibodies, anti-RNA antibodies, anti-hormone antibodies, anti-hypophysiotropic peptides, antibodies against non-natural antigens, anti-anterior pituitary hormone antibodies, anti-posterior pituitary hormone antibodies, anti-venom antibodies, anti-tumor marker antibodies, antibodies directed against epitopes associated with infectious disease, including, anti-
10 viral, anti-bacterial, anti-protozoal, anti-fungal, anti-parasitic, anti-receptor, anti-lipid, anti-phospholipid, anti-growth factor, anti-cytokine, anti-monokine, anti-idiotype, and anti-accessory (presentation) protein antibodies. Antibodies made with the present invention, as well as light chains or heavy chains, may also be used to inhibit enzyme activity.

15   Antibodies that may be produced using the present invention include, but are not limited to, antibodies made against the following proteins: Bovine γ-Globulin, Serum; Bovine IgG, Plasma; Chicken γ-Globulin, Serum; Human γ-Globulin, Serum; Human IgA, Plasma; Human IgA₁, Myeloma; Human IgA₂, Myeloma; Human IgA₂, Plasma; Human IgD, Plasma; Human IgE, Myeloma; Human IgG, Plasma; Human
20 IgG, Fab Fragment, Plasma; Human IgG, F(ab')₂ Fragment, Plasma; Human IgG, Fc Fragment, Plasma; Human IgG₁, Myeloma; Human IgG₂, Myeloma; Human IgG₃, Myeloma; Human IgG₄, Myeloma; Human IgM, Myeloma; Human IgM, Plasma; Human Immunoglobulin, Light Chain κ, Urine; Human Immunoglobulin, Light Chains κ and λ, Plasma; Mouse γ-Globulin, Serum; Mouse IgG, Serum; Mouse IgM,
25 Myeloma; Rabbit γ-Globulin, Serum; Rabbit IgG, Plasma; and Rat γ-Globulin, Serum. In one embodiment, the transposon-based vector comprises the coding sequence of light and heavy chains of a murine monoclonal antibody that shows specificity for human seminoprotein (GenBank Accession numbers AY129006 and AY129304 for the light and heavy chains, respectively).
30   A further non-limiting list of antibodies that recognize other antibodies is as follows: Anti-Chicken IgG, heavy (H) & light (L) Chain Specific (Sheep); Anti-Goat γ-Globulin (Donkey); Anti-Goat IgG, Fc Fragment Specific (Rabbit); Anti-Guinea Pig γ-Globulin (Goat); Anti-Human Ig, Light Chain, Type κ Specific; Anti-Human Ig,

26

ATLLIB02 133492.1

Light Chain, Type λ Specific; Anti-Human IgA, α-Chain Specific (Goat); Anti-Human IgA, Fab Fragment Specific; Anti-Human IgA, Fc Fragment Specific; Anti-Human IgA, Secretory; Anti-Human IgE, ε-Chain Specific (Goat); Anti-Human IgE, Fc Fragment Specific; Anti-Human IgG, Fc Fragment Specific (Goat); Anti-Human IgG, γ-Chain Specific (Goat); Anti-Human IgG, Fc Fragment Specific; Anti-Human IgG, Fd Fragment Specific; Anti-Human IgG, H & L Chain Specific (Goat); Anti-Human IgG$_1$, Fc Fragment Specific; Anti-Human IgG$_2$, Fc Fragment Specific; Anti-Human IgG$_2$, Fd Fragment Specific; Anti-Human IgG$_3$, Hinge Specific; Anti-Human IgG$_4$, Fc Fragment Specific; Anti-Human IgM, Fc Fragment Specific; Anti-Human IgM, μ-Chain Specific; Anti-Mouse IgE, ε-Chain Specific; Anti-Mouse γ-Globulin (Goat); Anti-Mouse IgG, γ-Chain Specific (Goat); Anti-Mouse IgG, γ-Chain Specific (Goat) F(ab')$_2$ Fragment; Anti-Mouse IgG, H & L Chain Specific (Goat); Anti-Mouse IgM, μ-Chain Specific (Goat); Anti-Mouse IgM, H & L Chain Specific (Goat); Anti-Rabbit γ-Globulin (Goat); Anti-Rabbit IgG, Fc Fragment Specific (Goat); Anti-Rabbit IgG, H & L Chain Specific (Goat); Anti-Rat γ-Globulin (Goat); Anti-Rat IgG, H & L Chain Specific; Anti-Rhesus Monkey γ-Globulin (Goat); and, Anti-Sheep IgG, H & L Chain Specific.

Another non-limiting list of the antibodies that may be produced using the present invention is provided in product catalogs of companies such as Phoenix Pharmaceuticals, Inc. (www.phoenixpeptide.com; 530 Harbor Boulevard, Belmont, CA), Peninsula Labs San Carlos CA, SIGMA, St.Louis, MO www.sigma-aldrich.com, Cappel ICN, Irvine, California, www.icnbiomed.com, and Calbiochem, La Jolla, California, www.calbiochem.com, which are all incorporated herein by reference in their entirety. The polynucleotide sequences encoding these antibodies may be obtained from the scientific literature, from patents, and from databases such as GenBank. Alternatively, one of ordinary skill in the art may design the polynucleotide sequence to be incorporated into the genome by choosing the codons that encode for each amino acid in the desired antibody. Antibodies made by the transgenic animals of the present invention include antibodies that may be used as therapeutic reagents, for example in cancer immunotherapy against specific antigens, as diagnostic reagents and as laboratory reagents for numerous applications including immunoneutralization, radioimmunoassay, western blots, dot blots, ELISA, immunoprecipitation and immunoaffinity columns. Some of these antibodies include,

27

ATL.LIB02 1334S2.1

but are not limited to, antibodies which bind the following ligands: adrenomedulin, amylin, calcitonin, amyloid, calcitonin gene-related peptide, cholecystokinin, gastrin, gastric inhibitory peptide, gastrin releasing peptide, interleukin, interferon, cortistatin, somatostatin, endothelin, sarafotoxin, glucagon, glucagon-like peptide, insulin, atrial

5   natriuretic peptide, BNP, CNP, neurokinin, substance P, leptin, neuropeptide Y, melanin concentrating hormone, melanocyte stimulating hormone, orphanin, endorphin, dynorphin, enkephalin, enkephalin, leumorphin, peptide F, PACAP, PACAP-related peptide, parathyroid hormone, urocortin, corticotrophin releasing hormone, PHM, PHI, vasoactive intestinal polypeptide, secretin, ACTH, angiotensin,

10   angiostatin, bombesin, endostatin, bradykinin, FMRF amide, galanin, gonadotropin releasing hormone (GnRH) associated peptide, GnRH, growth hormone releasing hormone, inhibin, granulocyte-macrophage colony stimulating factor (GM-CSF), motilin, neurotensin, oxytocin, vasopressin, osteocalcin, pancreastatin, pancreatic polypeptide, peptide YY, proopiomelanocortin, transforming growth factor, vascular

15   endothelial growth factor, vesicular monoamine transporter, vesicular acetylcholine transporter, ghrelin, NPW, NPB, C3d, prokinetican, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, prolactin, growth hormone, beta-lipotropin, melatonin, kallikriens, kinins, prostaglandins, erythropoietin, p146 (SEQ ID NO:18 amino acid sequence, SEQ ID NO:19, nucleotide sequence), estrogen,

20   testosterone, corticosteroids, mineralocorticoids, thyroid hormone, thymic hormones, connective tissue proteins, nuclear proteins, actin, avidin, activin, agrin, albumin, and prohormones, propeptides, splice variants, fragments and analogs thereof.

          The following is yet another non-limiting of antibodies that can be produced by the methods of present invention: abciximab (ReoPro), abciximab anti-platelet

25   aggregation monoclonal antibody, anti-CD11a (hu1124), anti-CD18 antibody, anti-CD20 antibody, anti-cytomegalovirus (CMV) antibody, anti-digoxin antibody, anti-hepatitis B antibody, anti-HER-2 antibody, anti-idiotype antibody to GD3 glycolipid, anti-IgE antibody, anti-IL-2R antibody, antimetastatic cancer antibody (mAb 17-1A), anti-rabies antibody, anti-respiratory syncytial virus (RSV) antibody, anti-Rh

30   antibody, anti-TCR, anti-TNF antibody, anti-VEGF antibody and fab fragment thereof, rattlesnake venom antibody, black widow spider venom antibody, coral snake venom antibody, antibody against very late antigen-4 (VLA-4), C225 humanized antibody to EGF receptor, chimeric (human & mouse) antibody against TNFα,

antibody directed against GPIIb/IIIa receptor on human platelets, gamma globulin, anti-hepatitis B immunoglobulin, human anti-D immunoglobulin, human antibodies against *S aureus*, human tetanus immunoglobulin, humanized antibody against the epidermal growth receptor-2, humanized antibody against the α subunit of the

5 interleukin-2 receptor, humanized antibody CTLA4IG, humanized antibody to the IL-2 R α-chain, humanized anti-CD40-ligand monoclonal antibody (5c8), humanized mAb against the epidermal growth receptor-2, humanized mAb to rous sarcoma virus, humanized recombinant antibody (IgG1k) against respiratory syncytial virus (RSV), lymphocyte   immunoglobulin   (anti-thymocyte   antibody),   lymphocyte

10 immunoglobulin, mAb against factor VII, MDX-210 bi-specific antibody against HER-2, MDX-22, MDX-220 bi-specific antibody against TAG-72 on tumors, MDX-33 antibody to FcγR1 receptor, MDX-447 bi-specific antibody against EGF receptor, MDX-447 bispecific humanized antibody to EGF receptor, MDX-RA immunotoxin (ricin A linked) antibody, Medi-507 antibody (humanized form of BTI-322) against

15 CD2 receptor on T-cells, monoclonal antibody LDP-02, muromonab-CD3(OKT3) antibody, OKT3 ("muromomab-CD3") antibody, PRO 542 antibody, ReoPro ("abciximab") antibody, and TNF-IgG fusion protein.

The antibodies prepared using the methods of the present invention may also be designed to possess specific labels that may be detected through means known to

20 one of ordinary skill in the art. The antibodies may also be designed to possess specific sequences useful for purification through means known to one of ordinary skill in the art. Specialty antibodies designed for binding specific antigens may also be made in transgenic animals using the transposon-based vectors of the present invention.

25 Production of a monoclonal antibody using the transposon-based vectors of the present invention can be accomplished in a variety of ways. In one embodiment, two vectors may be constructed: one that encodes the light chain, and a second vector that encodes the heavy chain of the monoclonal antibody. These vectors may then be incorporated into the genome of the target animal by methods disclosed herein. In an

30 alternative embodiment, the sequences encoding light and heavy chains of a monoclonal antibody may be included on a single DNA construct. For example, the coding sequence of light and heavy chains of a murine monoclonal antibody that show specificity for human seminoprotein can be expressed using transposon-based

constructs of the present invention (GenBank Accession numbers AY129006 and AY129304 for the light and heavy chains, respectively).

Further included in the present invention are proteins and peptides synthesized by the immune system including those synthesized by the thymus, lymph nodes,
5   spleen, and the gastrointestinal associated lymph tissues (GALT) system. The immune system proteins and peptides proteins that can be made in transgenic animals using the transposon-based vectors of the present invention include, but are not limited to, alpha-interferon, beta-interferon, gamma-interferon, alpha-interferon A, alpha-interferon 1, G-CSF, GM-CSF, interlukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6,
10   IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, TNF-α, and TNF-β. Other cytokines included in the present invention include cardiotrophin, stromal cell derived factor, macrophage derived chemokine (MDC), melanoma growth stimulatory activity (MGSA), macrophage inflammatory proteins 1 alpha (MIP-1 alpha), 2, 3 alpha, 3 beta, 4 and 5.

15   Lytic peptides such as p146 are also included in the desired molecules of the present invention. In one embodiment, the p146 peptide comprises an amino acid sequence of SEQ ID NO:19. The present invention also encompasses a transposon-based vector comprising a p146 nucleic acid comprising a polynucleotide sequence of SEQ ID NO:20.

20   Enzymes are another class of proteins that may be made through the use of the transposon-based vectors of the present invention. Such enzymes include but are not limited to adenosine deaminase, alpha-galactosidase, cellulase, collagenase, dnaseI, hyaluronidase, lactase, L-asparaginase, pancreatin, papain, streptokinase B, subtilisin, superoxide dismutase, thrombin, trypsin, urokinase, fibrinolysin, glucocerebrosidase
25   and plasminogen activator. In some embodiments wherein the enzyme could have deleterious effects, additional amino acids and a protease cleavage site are added to the carboxy end of the enzyme of interest in order to prevent expression of a functional enzyme. Subsequent digestion of the enzyme with a protease results in activation of the enzyme.

30   Extracellular matrix proteins are one class of desired proteins that may be made through the use of the present invention. Examples include but are not limited to collagen, fibrin, elastin, laminin, and fibronectin and subtypes thereof. Intracellular

proteins and structural proteins are other classes of desired proteins in the present invention.

Growth factors are another desired class of proteins that may be made through the use of the present invention and include, but are not limited to, transforming growth factor-α ("TGF-α"), transforming growth factor-β (TGF-β), platelet-derived growth factors (PDGF), fibroblast growth factors (FGF), including FGF acidic isoforms 1 and 2, FGF basic form 2 and FGF 4, 8, 9 and 10, nerve growth factors (NGF) including NGF 2.5s, NGF 7.0s and beta NGF and neurotrophins, brain derived neurotrophic factor, cartilage derived factor, growth factors for stimulation of the production of red blood cells, growth factors for stimulation of the production of white blood cells, bone growth factors (BGF), basic fibroblast growth factor, vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor (G-CSF), insulin like growth factor (IGF) I and II, hepatocyte growth factor, glial neurotrophic growth factor (GDNF), stem cell factor (SCF), keratinocyte growth factor (KGF), transforming growth factors (TGF), including TGFs alpha, beta, beta1, beta2, beta3, skeletal growth factor, bone matrix derived growth factors, bone derived growth factors, erythropoietin (EPO) and mixtures thereof.

Another desired class of proteins that may be made may be made through the use of the present invention include but are not limited to leptin, leukemia inhibitory factor (LIF), tumor necrosis factor alpha and beta, ENBREL, angiostatin, endostatin, thrombospondin, osteogenic protein-1, bone morphogenetic proteins 2 and 7, osteonectin, somatomedin-like peptide, and osteocalcin.

A non-limiting list of the peptides and proteins that may be made may be made through the use of the present invention is provided in product catalogs of companies such as Phoenix Pharmaceuticals, Inc. (www.phoenixpeptide.com; 530 Harbor Boulevard • Belmont, CA), Peninsula Labs San Carlos CA, SIGMA, St.Louis, MO www.sigma-aldrich.com, Cappel ICN, Irvine, California, www.icnbiomed.com, and Calbiochem, La Jolla, California, www.calbiochem.com. The polynucleotide sequences encoding these proteins and peptides of interest may be obtained from the scientific literature, from patents, and from databases such as GenBank. Alternatively, one of ordinary skill in the art may design the polynucleotide sequence to be incorporated into the genome by choosing the codons that encode for each amino acid in the desired protein or peptide.

31

Some of these desired proteins or peptides that may be made through the use of the present invention include but are not limited to the following: adrenomedulin, amylin, calcitonin, amyloid, calcitonin gene-related peptide, cholecystokinin, gastrin, gastric inhibitory peptide, gastrin releasing peptide, interleukin, interferon, cortistatin,

5   somatostatin, endothelin, sarafotoxin, glucagon, glucagon-like peptide, insulin, atrial natriuretic peptide, BNP, CNP, neurokinin, substance P, leptin, neuropeptide Y, melanin concentrating hormone, melanocyte stimulating hormone, orphanin, endorphin, dynorphin, enkephalin, leumorphin, peptide F, PACAP, PACAP-related peptide, parathyroid hormone, urocortin, corticotrophin releasing hormone, PHM,

10   PHI, vasoactive intestinal polypeptide, secretin, ACTH, angiotensin, angiostatin, bombesin, endostatin, bradykinin, FMRF amide, galanin, gonadotropin releasing hormone (GnRH) associated peptide, GnRH, growth hormone releasing hormone, inhibin, granulocyte-macrophage colony stimulating factor (GM-CSF), motilin, neurotensin, oxytocin, vasopressin, osteocalcin, pancreastatin, pancreatic polypeptide,

15   peptide YY, proopiomelanocortin, transforming growth factor, vascular endothelial growth factor, vesicular monoamine transporter, vesicular acetylcholine transporter, ghrelin, NPW, NPB, C3d, prokinetican, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, prolactin, growth hormone, beta-lipotropin, melatonin, kallikriens, kinins, prostaglandins, erythropoietin, p146 (SEQ ID NO:19,

20   amino acid sequence, SEQ ID NO:20, nucleotide sequence), thymic hormones, connective tissue proteins, nuclear proteins, actin, avidin, activin, agrin, albumin, and prohormones, propeptides, splice variants, fragments and analogs thereof.

Other desired proteins that may be made by the transgenic animals of the present invention include bacitracin, polymixin b, vancomycin, cyclosporine, anti-

25   RSV antibody, alpha-1 antitrypsin (AAT), anti-cytomegalovirus antibody, anti-hepatitis antibody, anti-inhibitor coagulant complex, anti-rabies antibody, anti-Rh(D) antibody, adenosine deaminase, anti-digoxin antibody, antivenin crotalidae (rattlesnake venom antibody), antivenin latrodectus (black widow spider venom antibody), antivenin micrurus (coral snake venom antibody), aprotinin, corticotropin

30   (ACTH), diphtheria antitoxin, lymphocyte immune globulin (anti-thymocyte antibody), protamine, thyrotropin, capreomycin, α-galactosidase, gramicidin, streptokinase, tetanus toxoid, tyrothricin, IGF-1, proteins of varicella vaccine, anti-TNF antibody, anti-IL-2r antibody, anti-HER-2 antibody, OKT3 ("muromonab-

32

CD3") antibody, TNF-IgG fusion protein, ReoPro ("abciximab") antibody, ACTH fragment 1-24, desmopressin, gonadotropin-releasing hormone, histrelin, leuprolide, lypressin, nafarelin, peptide that binds GPIIb/GPIIIa on platelets (integrilin), goserelin, capreomycin, colistin, anti-respiratory syncytial virus, lymphocyte immune globulin (Thymoglovin, Atgam), panorex, alpha-antitrypsin, botulinin, lung surfactant protein, tumor necrosis receptor-IgG fusion protein (enbrel), gonadorelin, proteins of influenza vaccine, proteins of rotavirus vaccine, proteins of haemophilus b conjugate vaccine, proteins of poliovirus vaccine, proteins of pneumococcal conjugate vaccine, proteins of meningococcal C vaccine, proteins of influenza vaccine, megakaryocyte growth and development factor (MGDF), neuroimmunophilin ligand-A (NIL-A), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), leptin (native), leptin B, leptin C, IL-1RA (interleukin-1RA), R-568, novel erythropoiesis-stimulating protein (NESP), humanized mAb to rous sarcoma virus (MEDI-493), glutamyl-tryptophan dipeptide IM862, LFA-3TIP immunosuppressive, humanized anti-CD40-ligand monoclonal antibody (5c8), gelsonin enzyme, tissue factor pathway inhibitor (TFPI), proteins of meningitis B vaccine, antimetastatic cancer antibody (mAb 17-1A), chimeric (human & mouse) mAb against TNFα, mAb against factor VII, relaxin, capreomycin, glycopeptide (LY333328), recombinant human activated protein C (rhAPC), humanized mAb against the epidermal growth receptor-2, altepase, anti-CD20 antigen, C2B8 antibody, insulin-like growth factor-1, atrial natriuretic peptide (anaritide), tenectaplase, anti-CD11a antibody (hu 1124), anti-CD18 antibody, mAb LDP-02, anti-VEGF antibody, fab fragment of anti-VEGF Ab, APO2 ligand (tumor necrosis factor-related apoptosis-inducing ligand), rTGF-β (transforming growth factor-β), alpha-antitrypsin, ananain (a pineapple enzyme), humanized mAb CTLA4IG, PRO 542 (mAb), D2E7 (mAb), calf intestine alkaline phosphatase, α-L-iduronidase, α-L-galactosidase (humanglutamic acid decarboxylase, acid sphingomyelinase, bone morphogenetic protein-2 (rhBMP-2), proteins of HIV vaccine, T cell receptor (TCR) peptide vaccine, TCR peptides, V beta 3 and V beta 13.1. (IR502), (IR501), BI 1050/1272 mAb against very late antigen-4 (VLA-4), C225 humanized mAb to EGF receptor, anti-idiotype antibody to GD3 glycolipid, antibacterial peptide against *H. pylori*, MDX-447 bispecific humanized mAb to EGF receptor, anti-cytomegalovirus (CMV), Medi-491 B19 parvovirus vaccine, humanized recombinant mAb (IgG1k) against respiratory syncytial virus (RSV), urinary tract

33

infection vaccine (against "pili" on *Escherechia coli* strains), proteins of lyme disease vaccine against *B. burgdorferi* protein (DbpA), proteins of Medi-501 human papilloma virus-11 vaccine (HPV), *Streptococcus pneumoniae* vaccine, Medi-507 mAb (humanized form of BTI-322) against CD2 receptor on T-cells, MDX-33 mAb
5   to FcyR1 receptor, MDX-RA immunotoxin (ricin A linked) mAb, MDX-210 bi-specific mAb against HER-2, MDX-447 bi-specific mAb against EGF receptor, MDX-22, MDX-220 bi-specific mAb against TAG-72 on tumors, colony-stimulating factor (CSF) (molgramostim), humanized mAb to the IL-2 R α-chain (basiliximab), mAb to IgE (IGE 025A), myelin basic protein-altered peptide (MSP771A),
10   humanized mAb against the epidermal growth receptor-2, humanized mAb against the α subunit of the interleukin-2 receptor, low molecular weight heparin, anti-hemophillic factor, and bactericidal/permeability-increasing protein (r-BPI).

         The peptides and proteins made using the present invention may be labeled using labels and techniques known to one of ordinary skill in the art. Some of these
15   labels are described in the "Handbook of Fluorescent Probes and Research Products", ninth edition, Richard P. Haugland (ed) Molecular Probes, Inc. Eugene, OR), which is incorporated herein in its entirety. Some of these labels may be genetically engineered into the polynucleotide sequence for the expression of the selected protein or peptide. The peptides and proteins may also have label-incorporation "handles"
20   incorporated to allow labeling of an otherwise difficult or impossible to label protein.

         It is to be understood that the various classes of desired peptides and proteins, as well as specific peptides and proteins described in this section may be modified as described below by inserting selected codons for desired amino acid substitutions into the gene incorporated into the transgenic animal.

25   The present invention may also be used to produce desired molecules other than proteins and peptides including, but not limited to, lipoproteins such as high density lipoprotein (HDL), HDL-Milano, and low density lipoprotein, lipids, carbohydrates, siRNA and ribozymes. In these embodiments, a gene of interest encodes a nucleic acid molecule or a protein that directs production of the desired
30   molecule.

         The present invention further encompasses the use of inhibitory molecules to inhibit endogenous (i.e., non-vector) protein production. These inhibitory molecules include antisense nucleic acids, siRNA and inhibitory proteins. In one embodiment, a

34

ATL_LIB02 1334921.1

transposon-based vector containing an ovalbumin DNA sequence, that upon
transcription forms a double stranded RNA molecule, is transfected into an animal
such as a bird and the bird's production of endogenous ovalbumin protein is reduced
by the interference RNA mechanism (RNAi). Additionally, inducible knockouts or
5   knockdowns of the endogenous protein may be created to achieve a reduction or
inhibition of endogenous protein production.

Modified Desired Proteins and Peptides

"Proteins", "peptides," "polypeptides" and "oligopeptides" are chains of amino
acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds
10   formed by a condensation reaction between the carboxyl group of the alpha carbon of
one amino acid and the amino group of the alpha carbon of another amino acid. The
terminal amino acid at one end of the chain (i.e., the amino terminal) has a free amino
group, while the terminal amino acid at the other end of the chain (i.e., the carboxy
terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated
15   N-terminus) refers to the free alpha-amino group on the amino acid at the amino
terminal of the protein, or to the alpha-amino group (imino group when participating
in a peptide bond) of an amino acid at any other location within the protein.
Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free
carboxyl group on the amino acid at the carboxy terminus of a protein, or to the
20   carboxyl group of an amino acid at any other location within the protein.

Typically, the amino acids making up a protein are numbered in order, starting
at the amino terminal and increasing in the direction toward the carboxy terminal of
the protein. Thus, when one amino acid is said to "follow" another, that amino acid is
positioned closer to the carboxy terminal of the protein than the preceding amino acid.
25   The term "residue" is used herein to refer to an amino acid (D or L) or an
amino acid mimetic that is incorporated into a protein by an amide bond. As such, the
amino acid may be a naturally occurring amino acid or, unless otherwise limited, may
encompass known analogs of natural amino acids that function in a manner similar to
the naturally occurring amino acids (i.e., amino acid mimetics). Moreover, an amide
30   bond mimetic includes peptide backbone modifications well known to those skilled in
the art.

Furthermore, one of skill will recognize that, as mentioned above, individual
substitutions, deletions or additions which alter, add or delete a single amino acid or a

ATLLIB02 1334492.1

small percentage of amino acids (typically less than about 5%, more typically less than about 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino

5   acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

10   4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

A conservative substitution is a substitution in which the substituting amino acid (naturally occurring or modified) is structurally related to the amino acid being

15   substituted, i.e., has about the same size and electronic properties as the amino acid being substituted. Thus, the substituting amino acid would have the same or a similar functional group in the side chain as the original amino acid. A "conservative substitution" also refers to utilizing a substituting amino acid which is identical to the amino acid being substituted except that a functional group in the side chain is

20   protected with a suitable protecting group.

Suitable protecting groups are described in Green and Wuts, "Protecting Groups in Organic Synthesis", John Wiley and Sons, Chapters 5 and 7, 1991, the teachings of which are incorporated herein by reference. Preferred protecting groups are those which facilitate transport of the peptide through membranes, for example, by

25   reducing the hydrophilicity and increasing the lipophilicity of the peptide, and which can be cleaved, either by hydrolysis or enzymatically (Ditter et al., 1968. J. Pharm. Sci. 57:783; Ditter et al., 1968. J. Pharm. Sci. 57:828; Ditter et al., 1969. J. Pharm. Sci. 58:557; King et al., 1987. Biochemistry 26:2294; Lindberg et al., 1989. Drug Metabolism and Disposition 17:311; Tunek et al., 1988. Biochem. Pharm. 37:3867;

30   Anderson et al., 1985 Arch. Biochem. Biophys. 239:538; and Singhal et al., 1987. FASEB J. 1:220). Suitable hydroxyl protecting groups include ester, carbonate and carbamate protecting groups. Suitable amine protecting groups include acyl groups and alkoxy or aryloxy carbonyl groups, as described above for N-terminal protecting

groups. Suitable carboxylic acid protecting groups include aliphatic, benzyl and aryl esters, as described below for C-terminal protecting groups. In one embodiment, the carboxylic acid group in the side chain of one or more glutamic acid or aspartic acid residues in a peptide of the present invention is protected, preferably as a methyl,

5   ethyl, benzyl or substituted benzyl ester, more preferably as a benzyl ester.

Provided below are groups of naturally occurring and modified amino acids in which each amino acid in a group has similar electronic and steric properties. Thus, a conservative substitution can be made by substituting an amino acid with another amino acid from the same group. It is to be understood that these groups are non-

10   limiting, i.e. that there are additional modified amino acids which could be included in each group.

Group I includes leucine, isoleucine, valine, methionine and modified amino acids having the following side chains: ethyl, n-propyl n-butyl. Preferably, Group I includes leucine, isoleucine, valine and methionine.

15   Group II includes glycine, alanine, valine and a modified amino acid having an ethyl side chain. Preferably, Group II includes glycine and alanine.

Group III includes phenylalanine, phenylglycine, tyrosine, tryptophan, cyclohexylmethyl glycine, and modified amino residues having substituted benzyl or phenyl side chains. Preferred substituents include one or more of

20   the following: halogen, methyl, ethyl, nitro, —NH$_2$, methoxy, ethoxy and — CN. Preferably, Group III includes phenylalanine, tyrosine and tryptophan.

Group IV includes glutamic acid, aspartic acid, a substituted or unsubstituted aliphatic, aromatic or benzylic ester of glutamic or aspartic acid (e.g., methyl, ethyl, n-propyl iso-propyl, cyclohexyl, benzyl or substituted benzyl),

25   glutamine, asparagine, —CO—NH— alkylated glutamine or asparagines (e.g., methyl, ethyl, n-propyl and iso-propyl) and modified amino acids having the side chain —(CH$_2$)$_3$—COOH, an ester thereof (substituted or unsubstituted aliphatic, aromatic or benzylic ester), an amide thereof and a substituted or unsubstituted N-alkylated amide thereof. Preferably, Group IV includes

30   glutamic acid, aspartic acid, methyl aspartate, ethyl aspartate, benzyl aspartate and methyl glutamate, ethyl glutamate and benzyl glutamate, glutamine and asparagine.

37

Group V includes histidine, lysine, ornithine, arginine, N-nitroarginine, ß-cycloarginine, γ-hydroxyarginine, N-amidinocitruline and 2-amino-4-guanidinobutanoic acid, homologs of lysine, homologs of arginine and homologs of ornithine. Preferably, Group V includes histidine, lysine,

5   arginine and ornithine. A homolog of an amino acid includes from 1 to about 3 additional or subtracted methylene units in the side chain.

Group VI includes serine, threonine, cysteine and modified amino acids having C1-C5 straight or branched alkyl side chains substituted with —OH or —SH, for example, —CH$_2$CH$_2$OH, —CH$_2$CH$_2$CH$_2$OH or -CH$_2$CH$_2$OHCH$_3$. Preferably,

10   Group VI includes serine, cysteine or threonine.

In another aspect, suitable substitutions for amino acid residues include "severe" substitutions. A "severe substitution" is a substitution in which the substituting amino acid (naturally occurring or modified) has significantly different size and/or electronic properties compared with the amino acid being substituted.

15   Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of severe substitutions of this type include the substitution of phenylalanine or cyclohexylmethyl glycine for alanine, isoleucine for

20   glycine, a D amino acid for the corresponding L amino acid, or —NH—CH[(—CH$_2$)$_5$—COOH]—CO— for aspartic acid. Alternatively, a functional group may be added to the side chain, deleted from the side chain or exchanged with another functional group. Examples of severe substitutions of this type include adding of valine, leucine or isoleucine, exchanging the carboxylic acid in the side chain of

25   aspartic acid or glutamic acid with an amine, or deleting the amine group in the side chain of lysine or ornithine. In yet another alternative, the side chain of the substituting amino acid can have significantly different steric and electronic properties that the functional group of the amino acid being substituted. Examples of such modifications include tryptophan for glycine, lysine for aspartic acid and —

30   (CH$_2$)$_4$COOH for the side chain of serine. These examples are not meant to be limiting.

In another embodiment, for example in the synthesis of a peptide 26 amino acids in length, the individual amino acids may be substituted according in the following manner:

AA_1 is serine, glycine, alanine, cysteine or threonine;

5   AA_2 is alanine, threonine, glycine, cysteine or serine;

AA_3 is valine, arginine, leucine, isoleucine, methionine, ornithine, lysine, N-nitroarginine, ß-cycloarginine, γ-hydroxyarginine, N-amidinocitruline or 2-amino-4-guanidinobutanoic acid;

AA_4 is proline, leucine, valine, isoleucine or methionine;

10   AA_5 is tryptophan, alanine, phenylalanine, tyrosine or glycine;

AA_6 is serine, glycine, alanine, cysteine or threonine;

AA_7 is proline, leucine, valine, isoleucine or methionine;

AA_8 is alanine, threonine, glycine, cysteine or serine;

AA_9 is alanine, threonine, glycine, cysteine or serine;

15   AA_{10} is leucine, isoleucine, methionine or valine;

AA_{11} is serine, glycine, alanine, cysteine or threonine;

AA_{12} is leucine, isoleucine, methionine or valine;

AA_{13} is leucine, isoleucine, methionine or valine;

AA_{14} is glutamine, glutamic acid, aspartic acid, asparagine, or a substituted or

20   unsubstituted aliphatic or aryl ester of glutamic acid or aspartic acid;

AA_{15} is arginine, N-nitroarginine, ß-cycloarginine, γ-hydroxy-arginine, N-amidinocitruline or 2-amino-4-guanidino-butanoic acid

AA_{16} is proline, leucine, valine, isoleucine or methionine;

AA_{17} is serine, glycine, alanine, cysteine or threonine;

25   AA_{18} is glutamic acid, aspartic acid, asparagine, glutamine or a substituted or unsubstituted aliphatic or aryl ester of glutamic acid or aspartic acid;

AA_{19} is aspartic acid, asparagine, glutamic acid, glutamine, leucine, valine, isoleucine, methionine or a substituted or unsubstituted aliphatic or aryl ester of glutamic acid or aspartic acid;

30   AA_{20} is valine, arginine, leucine, isoleucine, methionine, ornithine, lysine, N-nitroarginine, ß-cycloarginine, γ-hydroxyarginine, N-amidinocitruline or 2-amino-4-guanidinobutanoic acid;

AA_{21} is alanine, threonine, glycine, cysteine or serine;

39

AA_{22} is alanine, threonine, glycine, cysteine or serine;

AA_{23} is histidine, serine, threonine, cysteine, lysine or ornithine;

AA_{24} is threonine, aspartic acid, serine, glutamic acid or a substituted or unsubstituted aliphatic or aryl ester of glutamic acid or aspartic acid;

5   AA_{25} is asparagine, aspartic acid,, glutamic acid, glutamine, leucine, valine, isoleucine, methionine or a substituted or unsubstituted aliphatic or aryl ester of glutamic acid or aspartic acid; and

AA_{26} is cysteine, histidine, serine, threonine, lysine or ornithine.

It is to be understood that these amino acid substitutions may be made for
10   longer or shorter peptides than the 26 mer in the preceding example above, and for proteins.

In one embodiment of the present invention, codons for the first several N-terminal amino acids of the transposase are modified such that the third base of each codon is changed to an A or a T without changing the corresponding amino acid. It is
15   preferable that between approximately 1 and 20, more preferably 3 and 15, and most preferably between 4 and 12 of the first N-terminal codons of the gene of interest are modified such that the third base of each codon is changed to an A or a T without changing the corresponding amino acid. In one embodiment, the first ten N-terminal codons of the gene of interest are modified in this manner.

20   When several desired proteins, protein fragments or peptides are encoded in the gene of interest to be incorporated into the genome, one of skill in the art will appreciate that the proteins, protein fragments or peptides may be separated by a spacer molecule such as, for example, a peptide, consisting of one or more amino acids. Generally, the spacer will have no specific biological activity other than to join
25   the desired proteins, protein fragments or peptides together, or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the spacer may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity. The spacer may also be contained within a nucleotide sequence with a purification handle or be flanked by
30   proteolytic cleavage sites.

Such polypeptide spacers may have from about 5 to about 40 amino acid residues. The spacers in a polypeptide are independently chosen, but are preferably all the same. The spacers should allow for flexibility of movement in space and are

ATLLIB02 1334692.1

therefore typically rich in small amino acids, for example, glycine, serine, proline or alanine. Preferably, peptide spacers contain at least 60%, more preferably at least 80% glycine or alanine. In addition, peptide spacers generally have little or no biological and antigenic activity. Preferred spacers are (Gly-Pro-Gly-Gly)$_x$ (SEQ ID

5   NO:5) and (Gly$_4$-Ser)$_y$, wherein x is an integer from about 3 to about 9 and y is an integer from about 1 to about 8. Specific examples of suitable spacers include
(Gly-Pro-Gly-Gly)$_3$

SEQ ID NO:6   Gly Pro Gly Gly Gly Pro Gly Gly Gly Pro Gly Gly

(Gly$_4$-Ser)$_3$

10   SEQ ID NO:7   Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

or (Gly$_4$-Ser)$_4$

SEQ ID NO:8   Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser.

Nucleotide sequences encoding for the production of residues which may be

15   useful in purification of the expressed recombinant protein may also be built into the vector. Such sequences are known in the art and include the glutathione binding domain from glutathione S-transferase, polylysine, hexa-histidine or other cationic amino acids, thioredoxin, hemagglutinin antigen and maltose binding protein.

Additionally, nucleotide sequences may be inserted into the gene of interest to

20   be incorporated so that the protein or peptide can also include from one to about six amino acids that create signals for proteolytic cleavage. In this manner, if a gene is designed to make one or more peptides or proteins of interest in the transgenic animal, specific nucleotide sequences encoding for amino acids recognized by enzymes may be incorporated into the gene to facilitate cleavage of the large protein or peptide

25   sequence into desired peptides or proteins or both. For example, nucleotides encoding a proteolytic cleavage site can be introduced into the gene of interest so that a signal sequence can be cleaved from a protein or peptide encoded by the gene of interest. Nucleotide sequences encoding other amino acid sequences which display pH sensitivity or chemical sensitivity may also be added to the vector to facilitate

30   separation of the signal sequence from the peptide or protein of interest.

In one embodiment of the present invention, a TAG sequence is linked to the gene of interest. The TAG sequence serves three purposes: 1) it allows free rotation of the peptide or protein to be isolated so there is no interference from the native

protein or signal sequence, i.e. vitellogenin, 2) it provides a "purification handle" to isolate the protein using column purification, and 3) it includes a cleavage site to remove the desired protein from the signal and purification sequences. Accordingly, as used herein, a TAG sequence includes a spacer sequence, a purification handle and

5   a cleavage site. The spacer sequences in the TAG proteins contain one or more repeats shown in SEQ ID NO:25. A preferred spacer sequence comprises the sequence provided in SEQ ID NO:26. One example of a purification handle is the gp41 hairpin loop from HIV I. Exemplary gp41 polynucleotide and polypeptide sequences are provided in SEQ ID NO:24 and SEQ ID NO:23, respectively.

10   However, it should be understood that any antigenic region may be used as a purification handle, including any antigenic region of gp41. Preferred purification handles are those that elicit highly specific antibodies. Additionally, the cleavage site can be any protein cleavage site known to one of ordinary skill in the art and includes an enterokinase cleavage site comprising the Asp Asp Asp Asp Lys sequence (SEQ

15   ID NO:9) and a furin cleavage site. Constructs containing a TAG sequence are shown in Figures 2 and 3. In one embodiment of the present invention, the TAG sequence comprises a polynucleotide sequence of SEQ ID NO:22.

Methods of Administering Transposon-Based Vectors

In addition to the transposon-based vectors described above, the present

20   invention also includes methods of administering the transposon-based vectors to an animal, methods of producing a transgenic animal wherein a gene of interest is incorporated into the germline of the animal and methods of producing a transgenic animal wherein a gene of interest is incorporated into cells other than the germline cells of the animal. The transposon-based vectors of the present invention may be

25   administered to an animal via any method known to those of skill in the art, including, but not limited to, intraembryonic, intratesticular, intraoviduct, intraperitoneal, intraarterial, intravenous, topical, oral, nasal, and pronuclear injection methods of administration, or any combination thereof. The transposon-based vectors may also be administered within the lumen of an organ, into an organ, into a body cavity, into

30   the cerebrospinal fluid, through the urinary system or through any route to reach the desired cells.

The transposon-based vectors may be delivered through the vascular system to be distributed to the cells supplied by that vessel. For example, the compositions may

be placed in the artery supplying the ovary or supplying the fallopian tube to transfect cells in those tissues. In this manner, follicles could be transfected to create a germline transgenic animal. Alternatively, supplying the compositions through the artery leading to the oviduct would preferably transfect the tubular gland and

5   epithelial cells. Such transfected cells could manufacture a desired protein or peptide for deposition in the egg white. Administration of the compositions through the portal vein would target uptake and transformation of hepatic cells. Administration through the urethra and into the bladder would target the transitional epithelium of the bladder. Administration through the vagina and cervix would target the lining of the uterus.

10 Administration through the internal mammary artery would transfect secretory cells of the lactating mammary gland to perform a desired function, such as to synthesize and secrete a desired protein or peptide into the milk.

In a preferred embodiment, the animal is an egg-laying animal, and more preferably, an avian. In one embodiment, between approximately 1 and 50 µg,

15 preferably between 1 and 20 µg, and more preferably between 5 and 10 µg of transposon-based vector DNA is administered to the oviduct of a bird. Optimal ranges depending upon the type of bird and the bird's stage of sexual maturity. Intraoviduct administration of the transposon-based vectors of the present invention result in a PCR positive signal in the oviduct tissue, whereas intravascular

20 administration results in a PCR positive signal in the liver. In other embodiments, the transposon-based vector is administered to an artery that supplies the oviduct or the liver. These methods of administration may also be combined with any methods for facilitating transfection, including without limitation, electroporation, gene guns, injection of naked DNA, and use of dimethyl sulfoxide (DMSO).

25 The present invention includes a method of intraembryonic administration of a transposon-based vector to an avian embryo comprising the following steps: 1) incubating an egg on its side at room temperature for two hours to allow the embryo contained therein to move to top dead center (TDC); 2) drilling a hole through the shell without penetrating the underlying shell membrane; 3) injecting the embryo with

30 the transposon-based vector in solution; 4) sealing the hole in the egg; and 5) placing the egg in an incubator for hatching. Administration of the transposon-based vector can occur anytime between immediately after egg lay (when the embryo is at Stage X) and hatching. Preferably, the transposon-based vector is administered between 1 and

43

ATLLIB02 1334921

7 days after egg lay, more preferably between 1 and 2 days after egg lay. The transposon-based vectors may be introduced into the embryo in amounts ranging from about 5.0 µg to 10 pg, preferably 1.0 µg to 100 pg. Additionally, the transposon-based vector solution volume may be between approximately 1 µl to 75 µl in quail

5   and between approximately 1 µl to 500 µl in chicken.

The present invention also includes a method of intratesticular administration of a transposon-based vector including injecting a bird with a composition comprising the transposon-based vector, an appropriate carrier and an appropriate transfection reagent. In one embodiment, the bird is injected before sexual maturity, preferably

10   between approximately 4-14 weeks, more preferably between approximately 6-14 weeks and most preferably between 8-12 weeks old. In another embodiment, a mature bird is injected with a transposon-based vector an appropriate carrier and an appropriate transfection reagent. The mature bird may be any type of bird, but in one example the mature bird is a quail.

15   A bird is preferably injected prior to the development of the blood-testis barrier, which thereby facilitates entry of the transposon-based vector into the seminiferous tubules and transfection of the spermatogonia or other germline cells. At and between the ages of 4, 6, 8, 10, 12, and 14 weeks, it is believed that the testes of chickens are likely to be most receptive to transfection. In this age range, the

20   blood/testis barrier has not yet formed, and there is a relatively high number of spermatogonia relative to the numbers of other cell types, e.g., spermatids, etc. See J. Kumaran et al., 1949. Poultry Sci., 29:511-520. See also E. Oakberg, 1956. Am. J. Anatomy, 99:507-515; and P. Kluin et al., 1984. Anat. Embryol., 169:73-78.

The transposon-based vectors may be introduced into a testis in an amount

25   ranging from about 0.1 µg to 10 µg, preferably 1 µg to 10 µg, more preferably 3 µg to 10 µg. In a quail, about 5 µg is a preferred amount. In a chicken, about 5 µg to 10 µg per testis is preferred. These amounts of vector DNA may be injected in one dose or multiple doses and at one site or multiple sites in the testis. In a preferred embodiment, the vector DNA is administered at multiple sites in a single testis, both

30   testes being injected in this manner. In one embodiment, injection is spread over three injection sites: one at each end of the testis, and one in the middle. Additionally, the transposon-based vector solution volume may be between approximately 1 µl to 75 µl in quail and between approximately 1 µl to 500 µl in chicken. In a preferred

embodiment, the transposon-based vector solution volume may be between approximately 20 µl to 60 µl in quail and between approximately 50 µl to 250 µl in chicken. Both the amount of vector DNA and the total volume injected into each testis may be determined based upon the age and size of the bird.

5   According to the present invention, the transposon-based vector is administered in conjunction with an acceptable carrier and/or transfection reagent. Acceptable carriers include, but are not limited to, water, saline, Hanks Balanced Salt Solution (HBSS), Tris-EDTA (TE) and lyotropic liquid crystals. Transfection reagents commonly known to one of ordinary skill in the art that may be employed

10   include, but are not limited to, the following: cationic lipid transfection reagents, cationic lipid mixtures, polyamine reagents, liposomes and combinations thereof; SUPERFECT®, Cytofectene, BioPORTER®, GenePORTER®, NeuroPORTER®, and perfectin from Gene Therapy Systems; lipofectamine, cellfectin, DMRIE-C oligofectamine, and PLUS reagent from InVitrogen; Xtreme gene, fugene, DOSPER

15   and DOTAP from Roche; Lipotaxi and Genejammer from Strategene; and Escort from SIGMA. In one embodiment, the transfection reagent is SUPERFECT®. The ratio of DNA to transfection reagent may vary based upon the method of administration. In one embodiment, the transposon-based vector is administered intratesticularly and the ratio of DNA to transfection reagent can be from 1:1.5 to

20   1:15, preferably 1:2 to 1:10, all expressed as wt/vol. Transfection may also be accomplished using other means known to one of ordinary skill in the art, including without limitation electroporation, gene guns, injection of naked DNA, and use of dimethyl sulfoxide (DMSO).

        Depending upon the cell or tissue type targeted for transfection, the form of

25   the transposon-based vector may be important. Plasmids harvested from bacteria are generally closed circular supercoiled molecules, and this is the preferred state of a vector for gene delivery because of the ease of preparation. In some instances, transposase expression and insertion may be more efficient in a relaxed, closed circular configuration or in a linear configuration. In still other instances, a purified

30   transposase protein may be co-injected with a transposon-based vector containing the gene of interest for more immediate insertion. This could be accomplished by using a transfection reagent complexed with both the purified transposase protein and the transposon-based vector.

Testing for and Breeding Animals Carrying the Transgene

Following administration of a transposon-based vector to an animal, DNA is extracted from the animal to confirm integration of the gene of interest. Actual frequencies of integration are estimated both by comparative strength of the PCR

5　signal, and by histological evaluation of the tissues by quantitative PCR. Another method for estimating the rate of transgene insertion is the so-called primed in situ hybridization technique (PRINS). This method determines not only which cells carry a transgene of interest, but also into which chromosome the gene has inserted, and even what portion of the chromosome. Briefly, labeled primers are annealed to

10　chromosome spreads (affixed to glass slides) through one round of PCR, and the slides are then developed through normal in situ hybridization procedures. This technique combines the best features of in situ PCR and fluorescence in situ hybridization (FISH) to provide distinct chromosome location and copy number of the gene in question. The 28s rRNA gene will be used as a positive control for

15　spermatogonia to confirm that the technique is functioning properly. Using different fluorescent labels for the transgene and the 28s gene causes cells containing a transgene to fluoresce with two different colored tags.

Breeding experiments are also conducted to determine if germline transmission of the transgene has occurred. In a general bird breeding experiment

20　performed according to the present invention, each male bird was exposed to 2-3 different adult female birds for 3-4 days each. This procedure was continued with different females for a total period of 6-12 weeks. Eggs were collected daily for up to 14 days after the last exposure to the transgenic male, and each egg was incubated in a standard incubator. In the first series of experiments the resulting embryos were

25　examined for transgene presence at day 3 or 4 using PCR.

Any male producing a transgenic embryo was bred to additional females. Eggs from these females were incubated, hatched, and the chicks tested for the exogenous DNA. Any embryos that died were necropsied and examined directly for the transgene or protein encoded by the transgene, either by fluorescence or PCR.

30　The offspring that hatched and were found to be positive for the exogenous DNA were raised to maturity. These birds were bred to produce further generations of transgenic birds, to verify efficiency of the transgenic procedure and the stable

46

incorporation of the transgene into the germ line. The resulting embryos were examined for transgene presence at day 3 or 4 using PCR.

It is to be understood that the above procedure can be modified to suit animals other than birds and that selective breeding techniques may be performed to amplify gene copy numbers and protein output.

Production of Desired Proteins or Peptides in Egg White

In one embodiment, the transposon-based vectors of the present invention may be administered to a bird for production of desired proteins or peptides in the egg white. These trasnposon-based vectors preferably contain one or more of an ovalbumin promoter, an ovomucoid promoter, an ovalbumin signal sequence and an ovomucoid signal sequence. Oviduct-specific ovalbumin promoters are described in B. O'Malley et al., 1987. EMBO J., vol. 6, pp. 2305-12; A. Qiu et al., 1994. Proc. Nat. Acad. Sci. (USA), vol. 91, pp. 4451-4455; D. Monroe et al., 2000. Biochim. Biophys. Acta, 1517 (1):27-32; H. Park et al., 2000. Biochem., 39:8537-8545; and T. Muramatsu et al., 1996. Poult. Avian Biol. Rev., 6:107-123. Examples of transposon-based vectors designed for production of a desired protein in an egg white are shown in Figures 2 and 3.

Production of Desired Proteins or Peptides in Egg Yolk

The present invention is particularly advantageous for production of recombinant peptides and proteins of low solubility in the egg yolk. Such proteins include, but are not limited to, membrane-associated or membrane-bound proteins, lipophilic compounds; attachment factors, receptors, and components of second messenger transduction machinery. Low solubility peptides and proteins are particularly challenging to produce using conventional recombinant protein production techniques (cell and tissue cultures) because they aggregate in water-based, hydrophilic environments. Such aggregation necessitates denaturation and re-folding of the recombinantly-produced proteins, which may deleteriously affect their structure and function. Moreover, even highly soluble recombinant peptides and proteins may precipitate and require denaturation and renaturation when produced in sufficiently high amounts in recombinant protein production systems. The present invention provides an advantageous resolution of the problem of protein and peptide solubility during production of large amounts of recombinant proteins.

ATLLIB02 133492.1

In one embodiment of the present invention, deposition of a desired protein into the egg yolk is accomplished by attaching a sequence encoding a protein capable of binding to the yolk vitellogenin receptor to a gene of interest that encodes a desired protein. This transposon-based vector can be used for the receptor-mediated uptake

5   of the desired protein by the oocytes. In a preferred embodiment, the sequence ensuring the binding to the vitellogenin receptor is a targeting sequence of a vitellogenin protein. The invention encompasses various vitellogenin proteins and their targeting sequences. In a preferred embodiment, a chicken vitellogenin protein targeting sequence is used, however, due to the high degree of conservation among

10   vitellogenin protein sequences and known cross-species reactivity of vitellogenin targeting sequences with their egg-yolk receptors, other vitellogenin targeting sequences can be substituted. One example of a construct for use in the transposon-based vectors of the present invention and for deposition of an insulin protein in an egg yolk is provided in SEQ ID NO:27. In this embodiment, the transposon-based

15   vector contains a vitellogenin promoter, a vitellogenin targeting sequence, a TAG sequence, a pro-insulin sequence and a synthetic polyA sequence. The present invention includes, but is not limited to, vitellogenin targeting sequences residing in the N-terminal domain of vitellogenin, particularly in lipovitellin I. In one embodiment, the vitellogenin targeting sequence contains the polynucleotide

20   sequence of SEQ ID NO:18.

In a preferred embodiment, the transposon-based vector contains a transposase gene operably-linked to a liver-specific promoter and a gene of interest operably-linked to a liver-specific promoter and a vitellogenin targeting sequence. Figure 4 shows an example of such a construct. In another preferred embodiment, the

25   transposon-based vector contains a transposase gene operably-linked to a constitutive promoter and a gene of interest operably-linked to a liver-specific promoter and a vitellogenin targeting sequence.

Isolation and Purification of Desired Protein or Peptide

For large-scale production of protein, an animal breeding stock that is

30   homozygous for the transgene is preferred. Such homozygous individuals are obtained and identified through, for example, standard animal breeding procedures or PCR protocols.

ATLLIB02 1334972.1

Once expressed, peptides, polypeptides and proteins can be purified according to standard procedures known to one of ordinary skill in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis, high performance liquid chromatography, immunoprecipitation and the like. Substantially pure compositions of about 50 to 99% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

In one embodiment of the present invention, the animal in which the desired protein is produced is an egg-laying animal. In a preferred embodiment of the present invention, the animal is an avian and a desired peptide, polypeptide or protein is isolated from an egg white. Egg white containing the exogenous protein or peptide is separated from the yolk and other egg constituents on an industrial scale by any of a variety of methods known in the egg industry. See, e.g., W. Stadelman et al. (Eds.), Egg Science & Technology, Haworth Press, Binghamton, NY (1995). Isolation of the exogenous peptide or protein from the other egg white constituents is accomplished by any of a number of polypeptide isolation and purification methods well known to one of ordinary skill in the art. These techniques include, for example, chromatographic methods such as gel permeation, ion exchange, affinity separation, metal chelation, HPLC, and the like, either alone or in combination. Another means that may be used for isolation or purification, either in lieu of or in addition to chromatographic separation methods, includes electrophoresis. Successful isolation and purification is confirmed by standard analytic techniques, including HPLC, mass spectroscopy, and spectrophotometry. These separation methods are often facilitated if the first step in the separation is the removal of the endogenous ovalbumin fraction of egg white, as doing so will reduce the total protein content to be further purified by about 50%.

To facilitate or enable purification of a desired protein or peptide, transposon-based vectors may include one or more additional epitopes or domains. Such epitopes or domains include DNA sequences encoding enzymatic or chemical cleavage sites including, but not limited to, an enterokinase cleavage site; the glutathione binding domain from glutathione S-transferase; polylysine; hexa-histidine or other cationic amino acids; thioredoxin; hemagglutinin antigen; maltose binding protein; a fragment of gp41 from HIV; and other purification epitopes or domains commonly known to one of skill in the art.

49

ATL.LIB02 1334921

In one representative embodiment, purification of desired proteins from egg white utilizes the antigenicity of the ovalbumin carrier protein and particular attributes of a TAG linker sequence that spans ovalbumin and the desired protein. The TAG sequence is particularly useful in this process because it contains 1) a highly antigenic epitope, a fragment of gp41 from HIV, allowing for stringent affinity purification, and, 2) a recognition site for the protease enterokinase immediately juxtaposed to the desired protein. In a preferred embodiment, the TAG sequence comprises approximately 50 amino acids. A representative TAG sequence is provided below.

Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Thr Thr Cys Ile Leu Lys Gly Ser Cys Gly Trp Ile Gly Leu Leu *Asp Asp Asp Asp Lys* (SEQ ID NO:22)

The underlined sequences were taken from the hairpin loop domain of HIV gp-41 (SEQ ID NO:23). Sequences in italics represent the cleavage site for enterokinase (SEQ ID NO:9). The spacer sequence upstream of the loop domain was made from repeats of (Pro Ala Asp Asp Ala) (SEQ ID NO:25) to provide free rotation and promote surface availability of the hairpin loop from the ovalbumin carrier protein.

Isolation and purification of a desired protein is performed as follows:

1. Enrichment of the egg white protein fraction containing ovalbumin and the transgenic ovalbumin-TAG-desired protein.

2. Size exclusion chromatography to isolate only those proteins within a narrow range of molecular weights (a further enrichment of step 1).

3. Ovalbumin affinity chromatography. Highly specific antibodies to ovalbumin will eliminate virtually all extraneous egg white proteins except ovalbumin and the transgenic ovalbumin-TAG-desired protein.

4. gp41 affinity chromatography using anti-gp41 antibodies. Stringent application of this step will result in virtually pure transgenic ovalbumin-TAG-desired protein.

5. Cleavage of the transgene product can be accomplished in at least one of two ways:

   a. The transgenic ovalbumin-TAG-desired protein is left attached to the gp41 affinity resin (beads) from step 4 and the protease enterokinase is

added. This liberates the transgene target protein from the gp41 affinity resin while the ovalbumin-TAG sequence is retained. Separation by centrifugation (in a batch process) or flow through (in a column purification), leaves the desired protein together with enterokinase in

5   solution. Enterokinase is recovered and reused.

    b. Alternatively, enterokinase is immobilized on resin (beads) by the addition of poly-lysine moieties to a non-catalytic area of the protease. The transgenic ovalbumin-TAG-desired protein eluted from the affinity column of step 4 is then applied to the protease resin. Protease

10   action cleaves the ovalbumin-TAG sequence from the desired protein and leaves both entities in solution. The immobilized enterokinase resin is recharged and reused.

    c. The choice of these alternatives is made depending upon the size and chemical composition of the transgene target protein.

15   6. A final separation of either of these two (5a or 5b) protein mixtures is made using size exclusion, or enterokinase affinity chromatography. This step allows for desalting, buffer exchange and/or polishing, as needed.

Cleavage of the transgene product (ovalbumin-TAG-desired protein) by enterokinase, then, results in two products: ovalbumin-TAG and the desired protein.

20   More specific methods for isolation using the TAG label is provided in the Examples. Some desired proteins may require additions or modifications of the above-described approach as known to one of ordinary skill in the art. The method is scaleable from the laboratory bench to pilot and production facility largely because the techniques applied are well documented in each of these settings.

25   It is believed that a typical chicken egg produced by a transgenic animal of the present invention will contain at least 0.001 mg, from about 0.001 to 1.0 mg, or from about 0.001 to 100.0 mg of exogenous protein, peptide or polypeptide, in addition to the normal constituents of egg white (or possibly replacing a small fraction of the latter).

30   One of skill in the art will recognize that after biological expression or purification, the desired proteins, fragments thereof and peptides may possess a conformation substantially different than the native conformations of the proteins, fragments thereof and peptides. In this case, it is often necessary to denature and

51

reduce protein and then to cause the protein to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

Production of Protein or Peptide in Milk

5   In addition to methods of producing eggs containing transgenic proteins or peptides, the present invention encompasses methods for the production of milk containing transgenic proteins or peptides. These methods include the administration of a transposon-based vector described above to a mammal. In one embodiment, the transposon-based vector contains a transposase operably-linked to a constitutive

10   promoter and a gene of interest operably-linked to mammary specific promoter. Genes of interest can include, but are not limited to antiviral and antibacterial proteins and immunoglobulins.

Treatment of Disease and Animal Improvement

       In addition to production and isolation of desired molecules, the transposon-

15   based vectors of the present invention can be used for the treatment of various genetic disorders. For example, one or more transposon-based vectors can be administered to a human or animal for the treatment of a single gene disorder including, but not limited to, Huntington's disease, alpha-1-antitrypsin deficiency Alzheimer's disease, various forms or breast cancer, cystic fibrosis, galactosemia, congenital

20   hypothyroidism, maple syrup urine disease, neurofibromatosis 1, phenylketonuria, sickle cell disease, and Smith-Lemli-Opitz (SLO/RSH) Syndrome. Other diseases caused by single gene disorders that may be treated with the present invention include, autoimmune diseases, shipping fever in cattle, mastitis, bacterial or viral diseases, alteration of skin pigment in animals. In these embodiments, the

25   transposon-based vector contains a non-mutated, or non-disease causing form of the gene known to cause such disorder. Preferably, the transposase contained within the transposase-based vector is operably linked to an inducible promoter such as a tissue-specific promoter such that the non-mutated gene of interest is inserted into a specific tissue wherein the mutated gene is expressed in vivo.

30   In one embodiment of the present invention, a transposon-based vector comprising a gene encoding proinsulin is administered to diabetic animals or humans for incorporation into liver cells in order to treat or cure diabetes. The specific incorporation of the proinsulin gene into the liver is accomplished by placing the

transposase gene under the control of liver-specific promoter, such as G6P. This approach is useful for treatment of both Type I and Type II diabetes. The G6P promoter has been shown to be glucose responsive (Arguad, D., et al. 1996. Diabetes 45:1563-1571), and thus, glucose-regulated insulin production is achieved using DNA constructs of the present invention. Integrating a proinsulin gene into liver cells circumvents the problem of destruction of pancreatic islet cells in the course of Type I diabetes.

In another embodiment, shortly after diagnosis of Type I diabetes, the cells of the immune system destroying pancreatic β-cells are selectively removed using the transposon-based vectors of the present invention, thus allowing normal β-cells to repopulate the pancreas.

For treatment of Type II diabetes, a transposon-based vector containing a proinsulin gene is specifically incorporated into the pancreas by placing the transposase gene under the control of a pancreas-specific promoter, such as an insulin promoter. In this embodiment, the vector is delivered to a diabetic animal or human via injection into an artery feeding the pancreas. For delivery, the vector is complexed with a transfection agent. The artery distributes the complex throughout the pancreas, where individual cells receive the vector DNA. Following uptake into the target cell, the insulin promoter is recognized by transcriptional machinery of the cell, the transposase encoded by the vector is expressed, and stable integration of the proinsulin gene occurs. It is expected that a small percentage of the transposon-based vector is transported to other tissues, and that these tissues are transfected. However, these tissues are not stably transfected and the proinsulin gene is not incorporated into the cells' DNA due to failure of these cells to activate the insulin promoter. The vector DNA is likely lost when the cell dies or degraded over time.

In other embodiments, one or more transposon-based vectors are administered to an avian for the treatment of a viral or bacterial infection/disease including, but not limited to, Colibacillosis (Coliform infections), Mycoplasmosis (CRD, Air sac, Sinusitis), Fowl Cholera, Necrotic Enteritis, Ulcerative Enteritis (Quail disease), Pullorum Disease, Fowl Typhoid, Botulism, Infectious Coryza, Erysipelas, Avian Pox, Newcastle Disease, Infectious Bronchitis, Quail Bronchitis, Lymphoid Leukosis, Marek's Disease (Visceral Leukosis), Infectious Bursal Disease (Gumboro). In these

53

embodiments, the transposon-based vectors may be used in a manner similar to traditional vaccines.

In still other embodiments, one or more transposon-based vectors are administered to an animal for the production of an animal with enhanced growth characteristics and nutrient utilization.

The transposon-based vectors of the present invention can be used to transform any animal cell, including but not limited to: cells producing hormones, cytokines, growth factors, or any other biologically active substance; cells of the immune system; cells of the nervous system; muscle (striatal, cardiac, smooth) cells; vascular system cells; endothelial cells; skin cells; mammary cells; and lung cells, including bronchial and alveolar cells. Transformation of any endocrine cell by a transposon-based vector is contemplated as a part of a present invention. In one aspect of the present invention, cells of the immune system may be the target for incorporation of a desired gene or genes encoding for production of antibodies. Accordingly, the thymus, bone marrow, beta lymphocytes (or B cells), gastrointestinal associated lymphatic tissue (GALT), Peyer's patches, bursa Fabricius, lymph nodes, spleen, and tonsil, and any other lymphatic tissue, may all be targets for administration of the compositions of the present invention.

The transposon-based vectors of the present invention can be used to modulate (stimulate or inhibit) production of any substance, including but not limited to a hormone, a cytokine, or a growth factor, by an animal or a human cell. Modulation of a regulated signal within a cell or a tissue, such as production of a second messenger, is also contemplated as a part of the present invention. Use of the transposon-based vectors of the present invention is contemplated for treatment of any animal or human disease or condition that results from underproduction (such as diabetes) or overproduction (such as hyperthyroidism) of a hormone or other endogenous biologically active substance. Use of the transposon-based vectors of the present invention to integrate nucleotide sequences encoding RNA molecules, such as anti-sense RNA or short interfering RNA, is also contemplated as a part of the present invention.

Additionally, the transposon-based vectors of the present invention may be used to provide cells or tissues with "beacons", such as receptor molecules, for binding of therapeutic agents in order to provide tissue and cell specificity for the

therapeutic agents. Several promoters and exogenous genes can be combined in one vector to produce progressive, controlled treatments from a single vector delivery.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the

5   contrary, it is to be clearly understood that resort may be had to various embodiments, modifications and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the invention.

10   EXAMPLE 1
Preparation of Transposon-Based Vector pTnMod

A vector was designed for inserting a desired coding sequence into the genome of eukaryotic cells, given below as SEQ ID NO:1. The vector of SEQ ID NO:1, termed pTnMod, was constructed and its sequence verified.

15   This vector employed a cytomegalovirus (CMV) promoter. A modified Kozak sequence (ACCATG) (SEQ ID NO:13) was added to the promoter. The nucleotide in the wobble position in nucleotide triplet codons encoding the first 10 amino acids of transposase was changed to an adenine (A) or thymine (T), which did not alter the amino acid encoded by this codon. Two stop codons were added and a synthetic

20   polyA was used to provide a strong termination sequence. This vector uses a promoter designed to be active soon after entering the cell (without any induction) to increase the likelihood of stable integration. The additional stop codons and synthetic polyA insures proper termination without read through to potential genes downstream.

25   The first step in constructing this vector was to modify the transposase to have the desired changes. Modifications to the transposase were accomplished with the primers High Efficiency forward primer (Hef) Altered transposase (ATS)-Hef 5' ATCTCGAGACCATGTGTGAACTTGATATTTTACATGATTCTCTTTACC   3' (SEQ ID NO:10) and Altered transposase- High efficiency reverse primer (Her) 5'

30   GATTGATCATTATCATAATTTCCCCAAAGCGTAACC 3' (SEQ ID NO:11, a reverse complement primer). In the 5' forward primer ATS-Hef, the sequence CTCGAG (SEQ ID NO:12) is the recognition site for the restriction enzyme Xho I, which permits directional cloning of the amplified gene. The sequence ACCATG

55

(SEQ ID NO:13) contains the Kozak sequence and start codon for the transposase and the underlined bases represent changes in the wobble position to an A or T of codons for the first 10 amino acids (without changing the amino acid coded by the codon). Primer ATS-Her (SEQ ID NO:11) contains an additional stop codon TAA in addition

5   to native stop codon TGA and adds a Bcl I restriction site, TGATCA (SEQ ID NO:14), to allow directional cloning. These primers were used in a PCR reaction with pTnLac (p defines plasmid, tn defines transposon, and lac defines the beta fragment of the lactose gene, which contains a multiple cloning site) as the template for the transposase and a FailSafe™ PCR System (which includes enzyme, buffers,

10   dNTP's, MgCl$_2$ and PCR Enhancer; Epicentre Technologies, Madison, WI). Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

15   Purified DNA was digested with restriction enzymes Xho I (5') and Bcl I (3') (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research).

       Plasmid gWhiz (Gene Therapy Systems, San Diego, CA) was digested with

20   restriction enzymes Sal I and BamH I (New England Biolabs), which are compatible with Xho I and Bcl I, but destroy the restriction sites. Digested gWhiz was separated on an agarose gel, the desired band excised and purified as described above. Cutting the vector in this manner facilitated directional cloning of the modified transposase (mATS) between the CMV promoter and synthetic polyA.

25   To insert the mATS between the CMV promoter and synthetic polyA in gWhiz, a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) was used and the ligation set up according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol.

30   Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37° C before being spread to LB (Luria-Bertani media (broth or agar)) plates supplemented with 100 μg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37° C and resulting colonies picked to

LB/amp broth for overnight growth at 37° C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size (approximately 6.4 kbp)

5   were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the transposase were the desired changes and no further changes or mutations occurred due to PCR amplification. For

10   sequencing, Perkin-Elmer's Big Dye Sequencing Kit was used. All samples were sent to the Gene Probes and Expression Laboratory (LSU School of Veterinary Medicine) for sequencing on a Perkin-Elmer Model 377 Automated Sequencer.

Once a clone was identified that contained the desired mATS in the correct orientation, primers CMVf-NgoM IV (5' TTGCCGGCATCAGATTGGCTAT (SEQ

15   ID NO:15); underlined bases denote NgoM IV recognition site) and Syn-polyA-BstE II (5' AGAGGTCACCGGGTCAATTCTTCAGCACCTGGTA (SEQ ID NO:16); underlined bases denote BstE II recognition site) were used to PCR amplify the entire CMV promoter, mATS, and synthetic polyA for cloning upstream of the transposon in pTnLac. The PCR was conducted with FailSafe™ as described above, purified

20   using the Zymo Clean and Concentrator kit, the ends digested with NgoM IV and BstE II (New England Biolabs), purified with the Zymo kit again and cloned upstream of the transposon in pTnLac as described below.

Plasmid pTnLac was digested with NgoM IV and BstE II to remove the ptac promoter and transposase and the fragments separated on an agarose gel. The band

25   corresponding to the vector and transposon was excised, purified from the agarose, and dephosphorylated with calf intestinal alkaline phosphatase (New England Biolabs) to prevent self-annealing. The enzyme was removed from the vector using a Zymo DNA Clean and Concentrator-5. The purified vector and CMVp/mATS/polyA were ligated together using a Stratagene T4 Ligase Kit and transformed into *E. coli* as

30   described above.

Colonies resulting from this transformation were screened (mini-preps) as describe above and clones that were the correct size were verified by DNA sequence

analysis as described above. The vector was given the name pTnMod (SEQ ID NO:1) and includes the following components:

Base pairs 1-130 are a remainder of F1(-) ori from pBluescriptII sk(-) (Stratagene), corresponding to base pairs 1-130 of pBluescriptII sk(-).

5   Base pairs 131 - 132 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 133 -1777 are the CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems), corresponding to bp 229-1873 of pGWiz. The CMV promoter was modified by the addition of an ACC sequence upstream of ATG.

10   Base pairs 1778-1779 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 1780 - 2987 are the coding sequence for the transposase, modified from Tn10 (GenBank accession J01829) by optimizing codons for stability of the transposase mRNA and for the expression of protein. More specifically, in each of the

15   codons for the first ten amino acids of the transposase, G or C was changed to A or T when such a substitution would not alter the amino acid that was encoded.

Base pairs 2988-2993 are two engineered stop codons.

Base pair 2994 is a residue from ligation of restriction enzyme sites used in constructing the vector.

20   Base pairs 2995 - 3410 are a synthetic polyA sequence taken from the pGWiz vector (Gene Therapy Systems), corresponding to bp 1922-2337 of 10 pGWiz.

Base pairs 3415 - 3718 are non-coding DNA that is residual from vector pNK2859.

Base pairs 3719 - 3761 are non-coding λ DNA that is residual from pNK2859.

25   Base pairs 3762 - 3831 are the 70 bp of the left insertion sequence recognized by the transposon Tn10.

Base pairs 3832-3837 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 3838 - 4527 are the multiple cloning site from pBluescriptII sk(20),

30   corresponding to bp 924-235 of pBluescriptII sk(-). This multiple cloning site may be used to insert any coding sequence of interest into the vector.

Base pairs 4528-4532 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 4533 - 4602 are the 70 bp of the right insertion sequence recognized by the transposon Tn10.

Base pairs 4603 - 4644 are non-coding λ DNA that is residual from pNK2859.

Base pairs 4645 - 5488 are non-coding DNA that is residual from pNK2859.

5   Base pairs 5489 - 7689 are from the pBluescriptll sk(-) base vector - (Stratagene, Inc.), corresponding to bp 761-2961 of pBluescriptll sk(-).

Completing pTnMod is a pBlueScript backbone that contains a colE I origin of replication and an antibiotic resistance marker (ampicillin).

It should be noted that all non-coding DNA sequences described above can be 10   replaced with any other non-coding DNA sequence(s). Missing nucleotide sequences in the above construct represent restriction site remnants.

All plasmid DNA was isolated by standard procedures. Briefly, *Escherichia coli* containing the plasmid was grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was 15   recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 μL of PCR-grade water and stored at -20°C until used.

EXAMPLE 2

20   *Preparation of Transposon-Based Vector pTnMod (CMV/Red)*

A vector was designed for inserting a reporter gene (DsRed) under the control of the CMV promoter into the genome of vertebrate cells given below as SEQ ID NO:2. The reporter gene chosen was the DsRed gene, driven by the immediate early cytomegalovirus promoter, to produce a plasmid called pTnCMV/DsRed. The DsRed 25   gene product is a red fluorescent protein from an IndoPacific sea anemone, Discosoma sp., which fluoresces bright red at 558 nm. It is to be understood that the reporter gene, i.e., the DsRed gene, is only one embodiment of the present invention and that any gene of interest may be inserted into the plasmid in place of the DsRed reporter gene in any Experiment described herein.

30   The vector of SEQ ID NO:2, named pTnMod (CMV/Red), was constructed, and its sequence verified by re-sequencing. SEQ ID NO:2, pTnMod (CMV/Red), includes the following components:

Base pairs 1-130 are a remainder of F1(-) on from pBluescriptII sk(-) (Stratagene), corresponding to bp 1-130 of pBluescriptII sk(-).

Base pairs 131 - 132 are a residue from ligation of restriction enzyme sites used in constructing the vector.

5   Base pairs 133 -1777 are the CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems, corresponding to bp 229-1873 of pGWiz.

Base pairs 1778-1779 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 1780 - 2987 are the coding sequence for the transposase, modified

10   from Tn10 (GenBank accession J01829) by optimizing codons as discussed above.

Base pairs 2988-2993 are two engineered stop codons.

Base pair 2994 is a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 2995 - 3410 are a synthetic polyA sequence taken from the pGWiz

15   vector (Gene Therapy Systems), corresponding to bp 1922-2337 of pGWiz.

Base pairs 3415 - 3718 are non-coding DNA that is residual from vector pNK2859.

Base pairs 3719 - 3761 are non-coding λ DNA that is residual from pNK2859.

Base pairs 3762 - 3831 are the 70 bp of the left insertion sequence recognized

20   by the transposon Tn10.

Base pairs 3832-3837 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 3838 - 4044 are part of the multiple cloning site from pBluescriptII sk(-), corresponding to bp 924-718 of pBluescriptII sk(-).

25   Base pairs 4045-4048 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 4049-5693 are the CMV promoter/enhancer, taken from vector pGWiz (Gene Therapy Systems), corresponding to bp 229-1873 of pGWiz.

Base pairs 5694-5701 are a residue from ligation of restriction enzyme sites

30   used in constructing the vector.

Base pairs 5702 - 6617 are the DsRed reporter coding sequence, including polyA sequence, from pDsRed1.1 (Clontech), corresponding to bp 77 - 992 of pDsRed1.1.

Base pairs 6618 - 7101 are part of the multiple cloning site from pBluescriptII sk(-), corresponding to bp 718-235 of pBluescriptII sk(-).

Base pairs 7102-7106 are a residue from ligation of restriction enzyme sites used in constructing the vector.

5 Base pairs 7107 - 7176 are the 70 bp of the right insertion sequence recognized by the transposon Tn10.

Base pairs 7177 - 7218 are non-coding λ DNA that is residual from pNK2859.

Base pairs 7219 - 8062 are non-coding DNA that is residual from pNK2859.

Base pairs 8063 - 10263 are from the pBluescriptII sk(-) base vector
10 (Stratagene, Inc.), corresponding to bp 761-2961 of pBluescriptII sk(-).

It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s).

EXAMPLE 3

15 *Preparation of Transposon-Based Vector pTnMod (Oval/Red) – Chicken*

A vector was designed for inserting a reporter gene (DsRed) under the control of the ovalbumin promoter, and including the ovalbumin signal sequence, into the genome of a bird. One version of this vector is given below as SEQ ID NO:3. The vector of SEQ ID NO:3, named pTnMod (Oval/Red) - Chicken, includes chicken
20 ovalbumin promoter and signal sequences.

SEQ ID NO:3, pTnMod (Oval/Red) - Chicken, includes the following components:

Base pairs 1-130 are a remainder of F1(-) on from pBluescriptII sk(-) (Stratagene), corresponding to bp 1-130 of pBluescriptII sk(-).

25 Base pairs 131 - 132 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 133 -1777 are the CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems, corresponding to bp 229-1873 of pGWiz.

Base pairs 1778-1779 are a residue from ligation of restriction enzyme sites
30 used in constructing the vector.

Base pairs 1780 - 2987 are the coding sequence for the transposase, modified from Tn10 (GenBank accession J01829) by optimizing codons as discussed above.

Base pairs 2988-2993 are two engineered stop codons.

Base pair 2994 is a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 2995 - 3410 are a synthetic polyA sequence taken from the pGWiz vector (Gene Therapy Systems), corresponding to bp 1922-2337 of pGWiz.

5   Base pairs 3415 --3718 are non-coding DNA that is residual from vector pNK2859.

Base pairs 3719 - 3761 are non-coding λ DNA that is residual from 10 pNK2859.

Base pairs 3762 - 3831 are the 70 bp of the left insertion sequence recognized 10   by the transposon Tn10.

Base pairs 3832-3837 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 3838 - 4044 are part of the multiple cloning site from pBluescriptII sk(-), corresponding to bp 924-718 of pBluescriptII sk(-).

15   Base pairs 4045-4049 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 4050 - 4951 contain upstream elements of the (including SDRE, steroid-dependent response element). See GenBank accession number J00895 M24999, bp 431-1332.   Base pairs 4952-4959 are a residue from ligation of 20   restriction enzyme sites used in constructing the vector.

Base pairs 4960 - 5112 are the chicken ovalbumin signal sequence (GenBank accession number J00895 M24999, bp 2996-3148).

Base pairs 5113-5118 are a residue from ligation of restriction enzyme sites used in constructing the vector.

25   Base pairs 5119 - 6011 are the DsRed reporter coding sequence, including polyA sequence, from pDsRed1.1 (Clontech), corresponding to bp 100 - 992 of pDsRed1.1.

Base pairs 6012-6017 are a residue from ligation of restriction enzyme sites used in constructing the vector.

30   Base pairs 6018 - 6056 are part of the multiple cloning site of the ZeroBlunt Topo cloning vector (Invitrogen), corresponding to bp 337-377 of ZeroBlunt.

Base pairs 6057-6062 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 6063 - 6495 are part of the multiple cloning site from pBluescriptll sk(-), corresponding to bp 667-235 of pBluescriptll sk(-).

Base pairs 6496-6500 are a residue from ligation of restriction enzyme sites used in constructing the vector.

5   Base pairs 6501 - 6570 are the 70 bp of the right insertion sequence recognized by the transposon Tn10.

Base pairs 6571 - 6612 are non-coding λ DNA that is residual from pNK2859.

Base pairs 6613 - 7477 are non-coding DNA that is residual from pNK2859.

Base pairs 7478 - 9678 are from the pBluescriptll sk(-) base vector
10   (Stratagene, Inc.), corresponding to bp 761-2961 of pBluescriptll sk(-).

It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s).

EXAMPLE 4

15   *Preparation of Transposon-Based Vector pTnMod(Oval/Red) – Quail*

A vector was designed for inserting a reporter gene (DsRed) under the control of the ovalbumin promoter, and including the ovalbumin signal sequence, into the genome of a bird given below as SEQ ID NO:4. The vector of SEQ ID NO:4, named pTnMod (Oval/Red) - Quail, has been constructed, and selected portions of the
20   sequence have been verified by re-sequencing.

SEQ ID NO:4, pTnMod (Oval/Red) - Quail, includes the following components:

Base pairs 1-130 are a remainder of F1(-) on from pBluescriptll sk(-) (Stratagene), corresponding to bp 1-130 of pBluescriptll sk(-).
25   Base pairs 131 - 132 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 133 - 1777 are the CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems), corresponding to bp 229-1873 of pGWiz.

Base pairs 1778-1779 are a residue from ligation of restriction enzyme sites
30   used in constructing the vector.

Base pairs 1780 - 2987 are the coding sequence for the transposase, modified from Tn10 (GenBank accession J01829) by optimizing codons as discussed above.

63

Base pairs 2988-2993 are two engineered stop codons. Base pair 2994 is a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 2995 - 3410 are a synthetic polyA sequence taken from the pGWiz vector (Gene Therapy Systems), corresponding to bp 1922-2337 of pGWiz.

5   Base pairs 3415 - 3718 are non-coding DNA that is residual from vector pNK2859.

Base pairs 3719 - 3761 are non-coding λ DNA that is residual from pNK2859.

Base pairs 3762 - 3831 are the 70 base pairs of the left insertion sequence recognized by the transposon Tn10.

10   Base pairs 3832-3837 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 3838 - 4044 are part of the multiple cloning site from pBluescriptII sk(-), corresponding to bp 924-718 of pBluescriptII sk(-).

Base pairs 4045-4049 are a residue from ligation of restriction enzyme sites
15   used in constructing the vector.

Base pairs 4050 - 4934 are the Japanese quail ovalbumin promoter (including SDRE, steroid-dependent response element). The Japanese quail ovalbumin promoter was isolated by its high degree of homology to the chicken ovalbumin promoter (GenBank accession number J00895 M24999, base pairs 431-1332). Some deletions
20   were noted in the quail sequence, as compared to the chicken sequence.

Base pairs 4935-4942 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 4943 - 5092 are the Japanese quail ovalbumin signal sequence. The quail signal sequence was isolated by its high degree of homology to the chicken
25   signal sequence (GenBank accession number J00895 M24999, base pairs 2996-3148). Some deletions were noted in the quail sequence, as compared to the chicken sequence.

Base pairs 5093-5098 are a residue from ligation of restriction enzyme sites used in constructing the vector.

30   Base pairs 5099 - 5991 are the DsRed reporter coding sequence, including polyA sequence, from pDsRed1.1 (Clontech), corresponding to bp 100 - 992 of pDsRed 1.1.

Base pairs 5992-5997 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 5998 - 6036 are part of the multiple cloning site of the ZeroBlunt Topo cloning vector (Invitrogen), corresponding to base pairs 337-377 of ZeroBlunt.

5   Base pairs 6037-6042 are a residue from ligation of restriction enzyme sites used in constructing the vector.

Base pairs 6043 - 6475 are part of the multiple cloning site from pBluescriptII sk(-), corresponding to bp 667-235 of pBluescriptII sk(-).

Base pairs 6476-6480 are a residue from ligation of restriction enzyme sites
10   used in constructing the vector.

Base pairs 6481 - 6550 are the 70 bp of the right insertion sequence recognized by the transposon Tn10.

Base pairs 6551 - 6592 are non-coding λ DNA that is residual from pNK2859.

Base pairs 6593 - 7457 are non-coding DNA that is residual from pNK2859.

15   Base pairs 7458 - 9658 are from the pBluescriptII sk(-) base vector (Stratagene, Inc.), corresponding to base pairs 761-2961 of pBluescriptII sk(-).

It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s).

20   EXAMPLE 5

Transfection of Stage X Japanese Quail Eggs with pTnMod(Oval/Red) – Quail via embryo injection

Transgenic Japanese quail were produced by transfecting Stage X embryos and the heritability of the transgene delivered by embryo transfection was established.
25   More specifically, fertile eggs were collected in the morning and placed at 15° C until enough were collected for injection, but were held no longer than 7 days. Stage X embryos (eggs) were assigned to one of two treatment groups. Before treatment, each egg was incubated on its side at room temperature for about 2 hours to allow the embryo to move to "top dead center" (TDC). Each egg was transfected by drilling a 1
30   mm hole (directly above the embryo) through the shell without penetrating the underlying shell membrane. A 0.5 ml syringe fitted with a 28 gauge needle was used to deliver DNA complexed to a transfecting reagent, i.e. SUPERFECT®, in a 50 µl volume. An adhesive disc was used to seal the hole and provide a label for treatment identification. After all eggs were transfected, they were set in an incubator with the
35   adhesive disc pointing upward for hatching.

65

Each bird that hatched was bled at one week of age, DNA was extracted from blood cells, and PCR was conducted using 28s primers as a positive control and primers specific to DsRed. Any bird that was negative was terminated, while positive birds were monitored to determine maintenance of the transgene. Birds consistently

5   positive were maintained until sexual maturity and bred. Positive male and female birds were mated. The eggs of mated hens were hatched and the resulting chicks, the G1 generation, were evaluated to determine if they were transgenic. All G1s resulting from this mating were bled and PCR conducted as described above.

Egg injection: Two treatment groups and one control group were used for this

10   experiment. Vector pTnMod (Oval/Red) in supercoiled form (Treatment 1) and in linear form (Treatment 2) were used to transfect 15 eggs per treatment. To obtain linear DNA for this experiment, pTnMod (Oval/Red) was digested with NgoM IV, column purified, and resuspended in TE buffer.

Each egg was injected with 0.75 µg of DNA complexed with SUPERFECT®

15   in a 1:3 ratio in a total injection volume of 50 µl Hank's Balanced Salt solution (HBSS) was used to bring the volume to 50 µl. The DNA Superfect mixture must be allowed to incubate (for complex formation) at room temperature for 10 minutes prior to injection and must be used within 40 minutes post initial mixing. Eggs were incubated as described above after injection.

20   Results: In the supercoiled injection group, 2 females and 1 male were identified as PCR positive using primers specific to the DsRed coding sequence. These birds were mated as described above. Blood was taken from the G1 chicks and PCR was conducted. The results showed that the transgene was incorporated into the gametes of these birds. The G1 chicks from these birds were examined on a weekly

25   basis until it was verified that the gene was not present or enough transgenic G1s were obtained to initiate a breeding flock of fully transgenic birds. Eggs from these G1 chicks expressed DsRed protein in the albumin portion of their eggs.

EXAMPLE 6

30   *Intratesticular Injection of Chickens with pTnMod(CMV/Red) (SEQ ID NO:2)*

Immature birds of different ages (4, 6, 8, 10, 12, and 14 weeks) were placed under anesthesia and injected in the testes with the construct pTnMod(CMV/Red). A saline solution containing 1-5 µg of purified DNA vector, mixed with SUPERFECT® transfecting reagent (Qiagen, Valencia, CA) in a 1:6 (wt:vol) ratio. The volume of

35   saline was adjusted so that the total volume injected into each testis was 150-200 µl, depending on the age and size of the bird. For the 4- and 6-week-old chickens, 1 µg DNA in 150 µl was injected in each testis, divided into three doses of 50 µl each. For

66

ATLLIB02 133492.1

the older birds, 200 µl total volume was injected, containing either 3 µg DNA (for 8-week-old birds) or 5 µg DNA (for older birds) per testis. First, one testis was surgically exposed prior to injection. After injection, the incision was sutured, and the sequence was repeated for the alternate testis.

5   From six to nine months post-surgery, weekly sperm samples were taken from each injected bird, as well as from control birds. Each sperm sample was evaluated for uptake and expression of the injected gene. Samples were evaluated by PCR on whole sperm, within one week after collection.

            Approximately 100 male white leghorn chickens, in groups of 5-26, at ages 4,
10   6, 8, 10, 12, and 14 weeks, were used as this is the age range in which it is expected that the testes are likely to be most "receptive." In this age range, the blood/testis barrier has not yet formed, and there is a relatively high number of spermatogonia relative to the numbers of other cell types, e.g., spermatids, etc. See J. Kumaran et al., 1949. Poultry Sci., vol. 29, pp. 511-520. See also E. Oakberg, 1956. Am. J. Anatomy,
15   vol. 99, pp. 507-515; and P. Kluin et al., 1984. Anat. EmbryoL, vol. 169, pp. 73-78.

            The experimental and control males were obtained from commercial sources at one day of age, and maintained in brooders until used. The male birds were housed in temperature-controlled spaces in individual standard caging as they approached maturity. They were given water and standard commercial feed ad lib. They were
20   kept initially in a 23:1 hour light/dark cycle, stepped down at approximately weekly intervals to a 15:8 hour light/dark cycle, as this regimen has been reported to optimize sexual maturity and fertility.

Surgical and DNA Injection Procedures

            At the appropriate ages, groups of individual males were starved overnight and
25   then subjected to transgene delivery by direct intratesticular injection of DNA by experienced animal surgeons. Each male was anesthetized with isoflurane via a simplified gas machine.

            Various devices and anesthesia machines have previously been described for administering isoflurane (and other gaseous anesthetics) to birds. See Alsage et al.,
30   Poultry Sci., 50:1876-1878 (1971); Greenlees et al., Am. J. Vet. Res., vol. 51, pp. 757-758 (1990). However, these prior techniques are somewhat cumbersome and complex to implement. A novel and much simpler system to administer isoflurane (or other gaseous) anesthesia was developed due to the deficiencies in the prior art, a

system that we found worked well on all ages of chicks. A standard nose cone was placed over the chick's head, similar to the system that has been used for decades to administer ether to mice. A plastic tube approximately 3.5 cm in diameter and 12 cm long was filled with cotton, into which was poured approximately 2 mL isoflurane
5   (Abbott Laboratories, Chicago). The chick's head was placed partially into the cylinder, and was held in place there intermittently throughout the surgery as required to maintain the proper plane of anesthesia, without overdosing.

Each anesthetized bird was positioned on its side on an animal board with cords tractioning the wings and feet to allow access to the testes area. The area was
10 swabbed with 0.5% chlorhexidine, and a 2 cm dorsolateral incision was made in the skin over the testis (similar to the procedure commonly used for caponization). A small-animal retractor was used to spread the last two ribs, exposing the testis. The DNA solution was then mixed with SUPERFECT® (Qiagen) according to the manufacturer's protocol, approximately a 1:6 wt/vol ratio, to a final concentration of
15 0.01 - 0.05 µg/µl. This resulted in 1 - 5 µg total DNA (in a 150-200 µl volume) being injected into each testis, spread over three injection sites: one at each end of the testis, and one in the middle.

The injection device was a standard 25 gauge, 1/2 inch (1.27 cm) hypodermic needle, attached to a 50, 100, or 200 µl syringe. Approximately 5 mm of the needle
20 tip was bent at a 90 degree angle, to facilitate insertion into the testes. Approximately 50 - 70 µl of the DNA-SUPERFECT® solution was injected into each of three sites per testis. The multiple injections were calculated to suffuse the DNA throughout the whole testis, the idea being to promote contact between DNA and spermatogonia as much as feasible. We estimated that our procedure resulted in the injection of about
25 100,000 DNA molecules per spermatogonium. The construct used in these tests was a highly potent constitutive modified CMV promoter, operatively linked to the dsRed gene as shown in SEQ ID NO:2.

Following injection, the incision was closed in two layers with 4-0 absorbable suture, and then the contralateral testis was similarly exposed and injected. Following
30 surgery, each bird was returned to its cage to recover. One hundred thirteen males were ultimately used in the experimental regimen to increase the overall likelihood of success, along with 4 control birds (16 weeks 20 old) subjected to sham surgery (with injections containing only the transfection reagent.

ATLLIB02 153492.1

Evaluation of Birds

Thus, a total of 113 white leghorn chickens were injected with the DNA vector in groups of 5-26 at varying ages. Fourteen birds were transformed at 4 weeks, 23 birds at 6 weeks; 26 birds at 8 weeks; 23 birds at 10 weeks; 5 birds at 12 weeks; and

5   22 birds at 14 weeks. Sixteen birds died before they could be sampled, so to date, 97 roosters have been sampled, plus the four controls. Birds were evaluated at 18-24 weeks of age for (a) potential transformation in the sperm, and (b) successful testis transfection. Sperm samples were obtained from each rooster by manual manipulation using standard techniques. The sperm were washed, and their DNA was

10   extracted following the techniques of G. Mann et al., 1993. J. Reprod. Fert., 99:505-12. The samples were then frozen until analyzed. Evaluation was conducted by PCR analysis to detect DNA integration into the sperm, or into any of the testicular cells. Additionally, selected testes were harvested at the end of the sperm sampling period.

Of 97 birds tested, at least 22 showed probable positive results. Positive

15   results were observed at all transformation ages, except for 4 weeks, which was not tested. At least two birds were confirmed positive by PCR of sperm, conducted four months after the initial injection. These results were transient in many cases, however since it was believed that the DsRed gene product used in these initial proof of concept experiments was toxic. Nevertheless, the positive PCR results presumptively

20   demonstrated that the transgene was incorporated into spermatogonia (before puberty), and that it was carried in transgenic sperm. Such sperm could then transmit the gene to subsequent generations, resulting in the production of true, germ-line transgenic "founder" birds.

To further confirm that the DNA had been incorporated into the sperm, and

25   that contaminating vector was not being detected from other sources, it was confirmed through PCR on sperm of experimental birds, and on positive and negative controls that the sperm of the experimental birds lacked DNA encoding the transposase. The design of the preferred transposon-based vector is such that the sequence encoding the transposase is contained in the vector, but is not incorporated into the transformed

30   chromosome. Thus, presence of the exogenous coding sequence, coupled with absence of the transposase gene, is strong evidence for incorporation of the exogenous coding sequence, or transgene.

ATLLIB02 133492.1

These results demonstrated proof of concept, as positive PCR results were obtained from the sperm of treated birds. Interpretation of these preliminary results was made more difficult by the fact that the modified CMV promoter used in the experiment was probably too "hot." As the DsRed product is not secreted from the cells, the product built up intracellularly to levels that were toxic, frequently killing the cells. Even this result, of course, means that the transformation was successful. The transgene could not have killed the cells otherwise.

In order to resolve to the problem with toxicity of the DsRed gene product, experiments were conducted using a different reporter gene operably linked to the ovalbumin promoter, so that the transgene was expressed in the egg white. These experiments are provided in Examples12-15 below.

EXAMPLE 7

Transfection of Male White Leghorn Chickens Using the Vector pTnMod(Oval/Red) – Quail (SEQ ID NO:4) via Testicular Injections

In further experiments conducted on leghorn chickens, it was demonstrated that chickens injected intratesticularly at 8, 10, 12, or 14 weeks of age, had, on average, approximately 40% positive sperm between 6 and 8 months after injection. In other experiments, successful transfection was achieved with chickens injected at 13 weeks of age.

Forty-nine white leghorn roosters approximately 8, 10, 12, or 14 weeks of age were obtained and housed. Birds were identified, wing banded, and assigned to a treatment group. If appropriate (based on testes size and vascularization), one testis was caponized and the entire DNA injection volume was delivered to the remaining testis. Thirty-two males received DNA injections of 5µg DNA/testis at a 1:3 ratio of DNA to SUPERFECT®. The remaining birds were used as controls. After injection, all birds were mated with at least 5 females and observed until sexual maturity and egg-laying began. All eggs collected prior to peak egg production (approximately 24 weeks of age for the hens) were incubated and candled to determine embryo presence. Any embryos identified were incubated to hatch to extract DNA, PCR was conducted, and transgene presence was determined.

Roosters positive for the pTnMod(Oval/Red) – Quail construct were kept to produce F1 offspring (eggs collected at peak production). Offspring from this hatch

were bled, DNA extracted from the blood, and PCR conducted using primers specific for the DsRed gene. It was determined that 77% of the offspring were transgenic.

EXAMPLE 8

5   *Transfection of Mature Male Japanese Quail using the vector pTnMod(Oval/Red) – Quail (SEQ ID NO:4) via Testicular Injections*

Twelve sexually mature males (at approximately 13 weeks of age) underwent surgery for testicular injection as described above for chickens. At 21-28 days of age, the birds were identified, leg banded, debeaked, and separated based on sex.

10   Injections comprised 5 µg/testes of the vector in concentrations 1:3 or 1:10 for SUPERFECT® or a 1:1 ratio with Mirrus. The study consisted of 3 treatment groups with 5 males in the 1:3 DNA:SUPERFECT® group, 3 males in the 1:10 DNA:SUPERFECT® group, and 4 males in the 1:1 Mirrus group. All surgeries were conducted in one day.

15   Any unincorporated DNA was allowed to clear from the testes by holding the birds for 19 days before mating with females. At 15 weeks of age, 2 age-matched females were housed with each treated male. The presence of the transfected DNA was determined in the fertilized eggs during the second week of egg lay. The subsequent eggs collected from parents producing positively identified transgenic

20   eggs were collected and stored until taken to hatch.

PCR performed on the sperm of quail injected at three months of age indicated successful incorporation of the DsRed transgene into the quail sperm.

EXAMPLE 9

25   *Transfection of Immature Male Japanese Quail using the vector pTnMod(Oval/Red) – Quail (SEQ ID NO:4) via Testicular Injections*

Approximately 450 quail eggs were set and hatched. At 21-28 days of age, the birds were identified, wingbanded, debeaked, and separated based on sex. At 4 weeks of age, 65 male birds underwent surgery and testicular injections as described above.

30   Injections comprised a control and 2 µg/testes of the vector in varying concentrations (0, 1/3, 1/5, and 1/10) of three different transfection reagents: 1) SUPERFECT®, 2) Mirus/Panvera and 3) Dosper. The study comprised 13 treatment groups with 5 males per group. One transfection reagent was administered per day.

71

At 7 weeks of age, 2 age-matched females were housed with each treated male. The presence of the transfected DNA was determined in the fertilized eggs during the second week of egg lay. The subsequent eggs collected from parents producing positively identified transgenic eggs were collected and stored until taken

5   to hatch. PCR performed on the sperm of quail injected at four and five weeks of age indicated successful incorporation of the DsRed transgene into the quail sperm.

EXAMPLE 10
Preparation of Transposon-Based Vector pTnMod(Oval/ENT TAG/p146/PA) –
10   *Chicken*

A vector is designed for inserting a p146 gene under the control of a chicken ovalbumin promoter, and a ovalbumin gene including an ovalbumin signal sequence, into the genome of a bird given below as SEQ ID NO:29.

Base pairs 1 - 130 are a remainder of F1(-) ori of pBluescriptII sk(-)
15   (Stratagene) corresponding to base pairs 1-130 of pBluescriptII sk(-).

Base pairs 133 – 1777 are a CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems) corresponding to base pairs 229-1873 of pGWiz.

Base pairs 1780 – 2987 are a transposase, modified from Tn10 (GenBank accession number J01829).

20   Base pairs 2988-2993 are an engineered stop codon.

Base pairs 2995 – 3410 are a synthetic polyA from pGWiz (Gene Therapy Systems) corresponding to base pairs 1922- 2337 of pGWiz.

Base pairs 3415 – 3718 are non coding DNA that is residual from vector pNK2859.

25   Base pairs 3719 – 3761 are λ DNA that is residual from pNK2859.

Base pairs 3762 – 3831 are the 70 base pairs of the left insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 3838 – 4044 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 924-718 of pBluescriptII sk(-).

30   Base pairs 4050 - 4951 are a chicken ovalbumin promoter (including SDRE) that corresponds to base pairs 431-1332 of the chicken ovalbumin promoter in GenBank Accession Number J00895 M24999.

ATLLIB02 1334921

Base pairs 4958 - 6115 are a chicken ovalbumin signal sequence and Ovalbumin gene that correspond to base pairs 66-1223 of GenBank Accession Number V00383.1 (The STOP codon being omitted).

Base pairs 6122 - 6271 are a TAG sequence containing a gp41 hairpin loop
5   from HIV I, an enterokinase cleavage site and a spacer (synthetic).

Base pairs 6272 - 6316 are a p146 sequence (synthetic) with 2 added stop codons.

Base pairs 6324 - 6676 are a synthetic polyadenylation sequence from pGWiz (Gene Therapy Systems) corresponding to base pairs 1920 - 2272of pGWiz.

10   Base pairs 6682 - 7114 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 667-235 of pBluescriptII sk(-).

Base pairs 7120- 7189 are the 70 base pairs of the right insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 7190 - 7231 are λ DNA that is residual from pNK2859.

15   Base pairs 7232 - 8096 are non coding DNA that is residual from pNK2859.

Base pairs 8097 - 10297 are pBlueScript sk(-) base vector (Stratagene, Inc.) corresponding to base pairs 761-2961of pBluescriptII sk(-).

It should be noted that all non-coding DNA sequences described above can be
20   replaced with any other non-coding DNA sequence(s). Missing nucleotide sequences in the above construct represent restriction site remnants.

EXAMPLE 11
Preparation of Transposon-Based Vector pTnMod(Oval/ENT TAG/p146/PA) – Quail

25   A vector is designed for inserting a p146 gene under the control of a quail ovalbumin promoter, and a ovalbumin gene including an ovalbumin signal sequence, into the genome of a bird given below as SEQ ID NO:30.

Base pairs 1 - 130 are a remainder of F1(-) ori of pBluescriptII sk(-) (Stratagene) corresponding to base pairs 1-130 of pBluescriptII sk(-).

30   Base pairs 133 - 1777 are a CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems) corresponding to base pairs 229-1873 of pGWiz.

Base pairs 1780 - 2987 are a transposase, modified from Tn10 (GenBank accession number J01829).

Base pairs 2988-2993 are an engineered stop codon.

Base pairs 2995 – 3410 are a synthetic polyA from pGWiz (Gene Therapy Systems) corresponding to base pairs 1922-2337 of pGWiz.

Base pairs 3415 – 3718 are non coding DNA that is residual from vector pNK2859.

5   Base pairs 3719 – 3761 are λ DNA that is residual from pNK2859.

Base pairs 3762 – 3831 are the 70 base pairs of the left insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 3838 – 4044 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 924-718 of pBluescriptII sk(-).

10   Base pairs 4050 - 4938 are the Japanese quail ovalbumin promoter (including SDRE, steroid-dependent response element). The Japanese quail ovalbumin promoter was isolated by its high degree of homology to the chicken ovalbumin promoter (GenBank accession number J00895 M24999, base pairs 431-1332).

Bp 4945 - 6092 are a quail ovalbumin signal sequence and ovalbumin gene

15   that corresponds to base pairs 54 – 1201 of GenBank accession number X53964.1. (The STOP codon being omitted).

Base pairs 6097 - 6246 are a TAG sequence containing a gp41 hairpin loop from HIV I, an enterokinase cleavage site and a spacer (synthetic).

Base pairs 6247 – 6291 are a p146 sequence (synthetic) with 2 added stop

20   codons.

Base pairs 6299 – 6651 are a synthetic polyadenylation sequence from pGWiz (Gene Therapy Systems) corresponding to base pairs 1920 - 2272of pGWiz.

Base pairs 6657 - 7089 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 667-235 of pBluescriptII sk(-).

25   Base pairs 7095- 7164 are the 70 base pairs of the right insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 7165 - 7206 are λ DNA that is residual from pNK2859.

Base pairs 7207 – 8071 are non coding DNA that is residual from pNK2859.

Base pairs 8072 - 10272 are pBlueScript sk(-) base vector (Stratagene, Inc.)

30   corresponding to base pairs 761-2961of pBluescriptII sk(-).

It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s). Missing nucleotide sequences in the above construct represent restriction site remnants.

5   EXAMPLE 12

Preparation of Transposon-Based Vector pTnMod(Oval/ENT TAG/ProIns/PA) – Chicken

A vector is designed for inserting a proinsulin gene under the control of a chicken ovalbumin promoter, and a ovalbumin gene including an ovalbumin signal

10   sequence, into the genome of a bird given below as SEQ ID NO:31.

Base pairs 1 - 130 are a remainder of FI(-) ori of pBluescriptII sk(-) (Stratagene) corresponding to base pairs 1-130 of pBluescriptII sk(-).

Base pairs 133 – 1777 are a CMV promoter/enhancer taken from vector

15   pGWiz (Gene Therapy Systems) corresponding to base pairs 229-1873 of pGWiz.

Base pairs 1780 – 2987 are a transposase, modified from Tn10 (GenBank accession number J01829).

Base pairs 2988-2993 are an engineered stop codon.

Base pairs 2995 – 3410 are a synthetic polyA from pGWiz (Gene Therapy

20   Systems) corresponding to base pairs 1922- 2337 of pGWiz.

Base pairs 3415 – 3718 are non coding DNA that is residual from vector pNK2859.

Base pairs 3719 – 3761 are λ DNA that is residual from pNK2859.

Base pairs 3762 – 3831 are the 70 base pairs of the left insertion sequence

25   (IS10) recognized by the transposon Tn10.

Base pairs 3838 – 4044 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 924-718 of pBluescriptII sk(-).

Base pairs 4050 - 4951 are a chicken ovalbumin promoter (including SDRE) that corresponds to base pairs 431-1332 of the chicken ovalbumin promoter in

30   GenBank Accession Number J00895 M24999.

Base pairs 4958 - 6115 are a chicken ovalbumin signal sequence and ovalbumin gene that correspond to base pairs 66-1223 of GenBank Accession Number V00383.1. (The STOP codon being omitted).

Base pairs 6122 - 6271 are a TAG sequence containing a gp41 hairpin loop from HIV I, an enterokinase cleavage site and a spacer (synthetic).

Base pairs 6272 – 6531 are a proinsulin gene.

Base pairs 6539 – 6891 are a synthetic polyadenylation sequence from pGWiz
5   (Gene Therapy Systems) corresponding to base pairs 1920 - 2272of pGWiz.

Base pairs 6897 - 7329 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 667-235 of pBluescriptII sk(-).

Base pairs 7335- 7404 are the 70 base pairs of the right insertion sequence (IS10) recognized by the transposon Tn10.
10 Base pairs 7405 - 7446 are λ DNA that is residual from pNK2859.

Base pairs 7447 – 8311 are non coding DNA that is residual from pNK2859.

Base pairs 8312 - 10512 are pBlueScript sk(-) base vector (Stratagene, Inc.) corresponding to base pairs 761-2961of pBluescriptII sk(-).

15 It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s). Missing nucleotide sequences in the above construct represent restriction site remnants.

EXAMPLE 13

20 *Preparation of Transposon-Based Vector pTnMod(Oval/ENT TAG/ProIns/PA) – Quail*

A vector is designed for inserting a proinsulin gene under the control of a chicken ovalbumin promoter, and a ovalbumin gene including an ovalbumin signal sequence, into the genome of a bird given below as SEQ ID NO:32.
25

Base pairs 1 -130 are a remainder of F1(-) ori of pBluescriptII sk(-) (Stratagene) corresponding to base pairs 1-130 of pBluescriptII sk(-).

Base pairs 133 – 1777 are a CMV promoter/enhancer taken from vector pGWiz (Gene Therapy Systems) corresponding to base pairs 229-1873 of pGWiz.
30 Base pairs 1780 – 2987 are a transposase, modified from Tn10 (GenBank accession number J01829).

Base pairs 2988-2993 are an engineered stop codon.

Base pairs 2995 – 3410 are a synthetic polyA from pGWiz (Gene Therapy Systems) corresponding to base pairs 1922- 2337 of pGWiz.

Base pairs 3415 – 3718 are non coding DNA that is residual from vector pNK2859.

5   Base pairs 3719 – 3761 are λ DNA that is residual from pNK2859.

Base pairs 3762 – 3831 are the 70 base pairs of the left insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 3838 – 4044 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 924-718 of pBluescriptII sk(-).

10   Base pairs 4050 - 4938 are the Japanese quail ovalbumin promoter (including SDRE, steroid-dependent response element). The Japanese quail ovalbumin promoter was isolated by its high degree of homology to the chicken ovalbumin promoter (GenBank accession number J00895 M24999, base pairs 431-1332). Some deletions were noted in the quail sequence, as compared to the chicken sequence.

15   Base pairs 4945 - 6092 are a quail ovalbumin signal sequence and ovalbumin gene that corresponds to base pairs 54 – 1201 of GenBank accession number X53964.1. (The STOP codon being omitted).

Base pairs 6093 - 6246 are a TAG sequence containing a gp41 hairpin loop from HIV I an enterokinase cleavage site and a spacer (synthetic).

20   Base pairs 6247 – 6507 are a proinsulin gene.

Base pairs 6514 – 6866 are a synthetic polyadenylation sequence from pGWiz (Gene Therapy Systems) corresponding to base pairs 1920 - 2272of pGWiz.

Base pairs 6867 - 7303 are a multiple cloning site from pBlueScriptII sk(-) corresponding to base pairs 667-235 of pBluescriptII sk(-).

25   Base pairs 7304- 7379 are the 70 base pairs of the right insertion sequence (IS10) recognized by the transposon Tn10.

Base pairs 7380 - 7421 are λ DNA that is residual from pNK2859.

Base pairs 7422 – 8286 are non coding DNA that is residual from pNK2859.

Base pairs 8287 - 10487 are pBlueScript sk(-) base vector (Stratagene, Inc.)

30   corresponding to base pairs 761-2961of pBluescriptII sk(-).

It should be noted that all non-coding DNA sequences described above can be replaced with any other non-coding DNA sequence(s). Missing nucleotide sequences in the above construct represent restriction site remnants.

5   EXAMPLE 14

Transfection of Immature Leghorn Roosters using a Transpson-based Vector containing a Proinsulin Gene via Testicular Injections

Vectors containing the elements Oval promoter/Oval gene/GP41 Enterokinase TAG/Proinsulin/Poly A (SEQ ID NO:31) and CMV promoter/Oval gene/GP41
10   Enterokinase TAG/Proinsulin/Poly A (SEQ ID NO:42) were each injected into the testes of 11 week old white leghorn roosters. These birds were held under normal conditions until sexual maturity was reached.

At the time of sexual maturity, each bird was handled and manipulated to obtain sperm. Sperm samples were collected in Hank's Buffered Salt Solution
15   (HBSS) and stored at either -20° C or 4° C until needed. DNA was extracted from sperm using a MoBio Ultra Clean DNA Bloodspin Kit (MoBio laboratories, Solana Beach CA). Fifty microliters of sperm was used in the DNA extraction protocol and the purified genomic DNA eluted in 100 µl of water. In each PCR reaction, approximately 0.5 – 0.75 µg of genomic DNA was used with primers anchored in the
20   entag-1 (5') and the synthetic polyA-2 (3'), which amplify a 685 bp fragment. Five of nine birds gave positive reactions for the presence of the appropriate vector construct. These birds were then mated with normal females.

Birds that did not yield positive results with PCR on the sperm were sacrificed, their testes removed, and DNA extracted using an approximately 25 mg
25   piece of tissue in a Qiagen DNEasy Tissue Kit; purified DNA was eluted in 200 µl water and PCR conducted as described above. Two of these birds gave a very strong, positive PCR reaction.

EXAMPLE 15

30   *Transfection of Japanese Quail using a Transposon-based Vector containing a Proinsulin Gene via Oviduct Injections*

Two experiments were conducted in Japanese quail using transpson-based vectors containing either Oval promoter/Oval gene/GP41 Enterokinase

TAG/Proinsulin/Poly A (SEQ ID NO:31) or CMV promoter/Oval gene/GP41 Enterokinase TAG/Proinsulin/Poly A (SEQ ID NO:42).

In the first experiment, the Oval promoter/Oval gene/GP41 Enterokinase TAG/Proinsulin/Poly A containing construct was injected into the oviduct of sexually

5   mature quail; three hens received 5 µg at a 1:3 Superfect ratio and three received 10 µg at a 1:3 Superfect ratio. As of the writing of the present application, at least one bird that received 10 µg of DNA was producing human proinsulin in egg white (other birds remain to be tested). This experiment indicates that 1) the DNA has been stable for at least 3 months; 2) protein levels are comparable to those observed with a

10   constitutive promoter such as the CMV promoter; and 3) sexually mature birds can be injected and results obtained without the need for cell culture.

In the second experiment, the transposon-based vector containing CMV promoter/Oval gene/GP41 Enterokinase TAG/Proinsulin/Poly A was injected into the oviduct of sexually immature Japanese quail. A total of 9 birds were injected. Of the

15   8 survivors, 3 produced human proinsulin in the white of their eggs for over 6 weeks. An ELISA assay described in detail below was developed to detect GP41 in the fusion peptide (Oval gene/GP41 Enterokinase TAG/Proinsulin) since the GP41 peptide sequence is unique and not found as part of normal egg white protein. In all ELISA assays, the same birds produced positive results and all controls worked as expected.

20   ELISA Procedure: Individual egg white samples were diluted in sodium carbonate buffer, pH 9.6, and added to individual wells of 96 well microtiter ELISA plates at a total volume of 0.1 ml. These plates were then allowed to coat overnight at 4°C. Prior to ELISA development, the plates were allowed warm to room temperature. Upon decanting the coating solutions and blotting away any excess,

25   non-specific binding of antibodies was blocked by adding a solution of phosphate buffered saline (PBS), 1% (w/v) BSA, and 0.05% (v/v) Tween 20 and allowing it to incubate with shaking for a minimum of 45 minutes. This blocking solution was subsequently decanted and replaced with a solution of the primary antibody (Goat Anti-GP41 TAG) diluted in fresh PBS/BSA/Tween 20. After a two hour period of

30   incubation with the primary antibody, each plate was washed with a solution of PBS and 0.05% Tween 20 in an automated plate washer to remove unbound antibody. Next, the secondary antibody, Rabbit anti-Goat Alkaline Phosphatase-conjugated, was diluted in PBS/BSA/Tween 20 and allowed to incubate 1 hour. The plates were then

subjected to a second wash with PBS/Tween 20. Antigen was detected using a solution of *p*-Nitrophenyl Phosphate in Diethanolamine Substrate Buffer for Alkaline Phosphatase and measuring the absorbance at 30 minutes and 1 hour.

5   EXAMPLE 16

Optimization of Intra-oviduct and Intra-ovarian Arterial Injections

Overall transfection rates of oviduct cells in a flock of chicken or quail hens are enhanced by synchronizing the development of the oviduct and ovary within the flock. When the development of the oviducts and ovaries are uniform across a group

10   of hens and when the stage of oviduct and ovarian development can be determined or predicted, timing of injections is optimized to transfect the greatest number of cells. Accordingly, oviduct development is synchronized as described below to ensure that a large and uniform proportion of oviduct secretory cells are transfected with the gene of interest.

15   Hens are treated with estradiol to stimulate oviduct maturation as described in Oka and Schimke (T. Oka and RT Schimke, J. Cell Biol., 41, 816 (1969)), Palmiter, Christensen and Schimke (J Biol. Chem. 245(4):833-845, 1970). Specifically, repeated daily injections of 1 mg estradiol benzoate are performed sometime before the onset of sexual maturation, a period ranging from 1 – 14 weeks of age. After a

20   stimulation period sufficient to maximize development of the oviduct, hormone treatment is withdrawn thereby causing regression in oviduct secretory cell size but not cell number. At an optimum time after hormone withdrawal, the oviducts of treated hens are injected with the transposon-based vector. Hens are subjected to additional estrogen stimulation after an optimized time during which the transposon-

25   based vector is taken up into oviduct secretory cells. Re-stimulation by estrogen activates the transposon mechanism of the transposon-based vector, causing the integration of the gene of interest into the host genome. Estrogen stimulation is then withdrawn and hens continue normal sexual development. If a developmentally regulated promoter such as the ovalbumin promoter is used, expression of the

30   transposon-based vector initiates in the oviduct at the time of sexual maturation. Intra-ovarian artery injection during this window allows for high and uniform transfection efficiencies of ovarian follicles to produce germ-line transfections and possibly oviduct expression.

Other means are also used to synchronize the development, or regression, of the oviduct and ovary to allow high and uniform transfection efficiencies. Alterations of lighting and/or feed regimens, for example, cause hens to 'molt' during which time the oviduct and ovary regress. Molting is used to synchronize hens for transfection, and may be used in conjunction with other hormonal methods to control regression and/or development of the oviduct and ovary.

EXAMPLE 17

Isolation of Human Proinsulin Using Anti-TAG Column Chromotography

A HiTrap NHS-activated 1 mL column (Amersham) was charged with a 30 amino acid peptide that contained the gp-41 epitope containing gp-41's native disulfide bond that stabilizes the formation of the gp-41 hairpin loop. The 30 amino acid gp41 peptide is provided as SEQ ID NO:23. Approximately 10 mg of the peptide was dissolved in coupling buffer (0.2 M NaHCO3, 0.5 M NaCl, pH 8.3 and the ligand was circulated on the column for 2 hours at room temperature at 0.5 mL/minute. Excess active groups were then deactivated using 6 column volumes of 0.5 M ethanolamine, 0.5 M NaCl, pH 8.3 and the column was washed alternately with 6 column volumes of acetate buffer (0.1 M acetate, 0.5 M NaCl, pH 4.0) and ethanolamine (above). The column was neutralized using 1 X PBS. The column was then washed with buffers to be used in affinity purification: 75 mM Tris, pH 8.0 and elution buffer, 100 mM glycine-HCl, 0.5 M NaCl, pH 2.7. Finally, the column was equilibrated in 75 mM Tris buffer, pH 8.0.

Antibodies to gp-41 were raised in goats by inoculation with the gp-41 peptide described above. More specifically, goats were inoculated, given a booster injection of the gp-41 peptide and then bled. Serum was harvested by centrifugation. Approximately 30 mL of goat serum was filtered to 0.45 uM and passed over a TAG column at a rate of 0.5 mL/min. The column was washed with 75 mM Tris, pH 8.0 until absorbance at 280 nm reached a baseline. Three column volumes (3 mL) of elution buffer (100 mM glycine, 0.5 M NaCl, pH 2.7) was applied, followed by 75 mM Tris buffer, pH 8.0, all at a rate of 0.5 mL/min. One milliliter fractions were collected. Fractions were collected into 200 uL 1 M Tris, pH 9.0 to neutralize acidic factions as rapidly as possible. A large peak eluted from the column, coincident with the application the elution buffer. Fractions were pooled. Analysis by SDS-PAGE

ATLLIB02 133492.1

showed a high molecular weight species that separated into two fragments under reducing condition, in keeping with the heavy and light chain structure of IgG.

Pooled antibody fractions were used to charge two 1 mL HiTrap NHS-activated columns, attached in series. Coupling was carried out in the same manner as
5   that used for charging the TAG column.

Isolation of Ovalbumin-TAG-Proinsulin from Egg White

Egg white from quail and chickens treated by intra-oviduct injection of the CMV-ovalbumin-TAG-proinsulin construct were pooled. Viscosity was lowered by subjecting the allantoid fluid to successively finer pore sizes using negative pressure
10   filtration, finishing with a 0.22 μM pore size. Through the process, egg white was diluted approximately 1:16. The clarified sample was loaded on the Anti-TAG column and eluted in the same manner as described for the purification of the anti-TAG antibodies. A peak of absorbance at 280 nm, coincident with the application of the elution buffer, indicated that protein had been specifically eluted from the Anti-
15   TAG column. Fractions containing the eluted peak were pooled for analysis.

The pooled fractions from the Anti-TAG affinity column were characterized by SDS-PAGE and western blot analysis. SDS-PAGE of the pooled fractions revealed a 60 kDal molecular weight band not present in control egg white fluid, consistent with the predicted molecular weight of the transgenic protein. Although some
20   contaminating bands were observed, the 60 kDal species was greatly enriched compared to the other proteins. An aliquot of the pooled fractions was cleaved overnight at room temperature with the protease, enterokinase. SDS-PAGE analysis of the cleavage product, revealed a band not present in the uncut material that co-migrated with a commercial human proinsulin positive control. Western blot analysis
25   showed specific binding to the 60 kDal species under non-reducing condition (which preserve the hairpin epitope of gp-41 by retaining the disulfide bond). Western analysis of the low molecular weight species that appeared upon cleavage with an anti-human proinsulin antibody, conclusively identified the cleaved fragment as human proinsulin.

30

EXAMPLE 18

Construction of a Transposon-based Transgene for the Expression of a Monoclonal Antibody

Production of a monoclonal antibody using transposon-based transgenic
5   methodology is accomplished in a variety of ways.

1) two vectors are constructed: one that encodes the light chain and a second vector that encodes the heavy chain of the monoclonal antibody. These vectors are then incorporated into the genome of the target animal by at least one of two methods: a) direct transfection of a single animal with both vectors (simultaneously or as separate
10   events); or, b) a male and a female of the species carry in their germline one of the vectors and then they are mated to produce progeny that inherit a copy of each.

2) the light and heavy chains are included on a single DNA construct, either separated by insulators and expression is governed by the same (or different) promoters, or by using a single promoter governing expression of both transgenes with the inclusion of
15   elements that permit separate transcription of both transgenes, such as an internal ribosome entry site.

The following example describes the production of a transposon-based DNA construct that contains both the coding region for a monoclonal light chain and a heavy chain on a single construct. Beginning with the vector pTnMod, the coding
20   sequences for the heavy and light chains are added, each preceded by an appropriate promoter and signal sequence. Using methods known to one skilled in the art, approximately 1 Kb of the proximal elements of the ovalbumin promoter are linked to the signal sequence of ovalbumin or some other protein secreted from the target tissue. Two copies of the promoter and signal sequence are added to the multiple
25   cloning site of pTnMod, leaving space and key restriction sites between them to allow the subsequent addition of the coding sequences of the light and heavy chains of the monoclonal antibody. Methods known to one skilled in the art allow the coding sequences of the light and heavy chains to be inserted in-frame for appropriate expression. For example, the coding sequence of light and heavy chains of a murine
30   monoclonal antibody that show specificity for human seminoprotein have recently been disclosed (GenBank Accession numbers AY129006 and AY129304 for the light and heavy chains, respectively). The light chain cDNA sequence is provided in SEQ

ID NO:34, whereas the cDNA of the heavy chain is reported as provided in SEQ ID NO:35.

Thus one skilled in the art can produce both the heavy and light chains of a monoclonal antibody in a single cell within a target tissue and species. If the modified cell contained normal posttranslational modification capabilities, the two chains would form their native configuration and disulfide attachments and be substrates for glycosylation. Upon secretion, then, the monoclonal antibody is accumulated, for example, in the egg white of a chicken egg, if the transgenes are expressed in the magnum of the oviduct.

It should also be noted that, although this example details production of a full-length murine monoclonal antibody, the method is quite capable of producing hybrid antibodies (e.g. a combination of human and murine sequences; 'humanized' monoclonal antibodies), as well as useful antibody fragments, known to one skilled in the art, such as Fab, Fc, F(ab) and Fv fragments. This method can be used to produce molecules containing the specific areas thought to be the antigen recognition sequences of antibodies (complementarity determining regions), linked, modified or incorporated into other proteins as desired.

EXAMPLE 19
Treatment of rats with a transposon-based vector for tissue-specific insulin gene incorporation

Rats are made diabetic by administering the drug streptozotocin (Zanosar; Upjohn, Kalamazoo, MI) at approximately 200 mg/kg. The rats are bred and maintained according to standard procedures. A transposon-based vector containing a proinsulin gene, an appropriate carrier, and, optionally, a transfection agent, are injected into rats' singhepatic (if using G6P) artery with the purpose of stable transformation. Incorporation of the insulin gene into the rat genome and levels of insulin expression are ascertained by a variety of methods known in the art. Blood and tissue samples from live or sacrificed animals are tested. A combination of PCR, Southern and Northern blots, *in-situ* hybridization and related nucleic acid analysis methods are used to determine incorporation of the vector-derived proinsulin DNA and levels of transcription of the corresponding mRNA in various organs and tissues of the rats. A combination of SDS-PAGE gels, Western Blot analysis, radioimmunoassay, and ELISA and other methods known to one of ordinary skill in

84

ATLLIB02 133492.1

the art are used to determine the presence of insulin and the amount produced. Additional transfections of the vector are used to increase protein expression if the initial amounts of the expressed insulin are not satisfactory, or if the level of expression tapers off. The physiological condition of the rats is closely examined

5   post-transfection to register positive or any negative effects of the gene therapy. Animals are examined over extended periods of time post-transfection in order to monitor the stability of gene incorporation and protein expression.

EXAMPLE 20

10

Exemplary Transposon-Based Vectors

The following example provides a description of various transposon-based vectors of the present invention and several constructs for insertion into the transposon-based vectors of the present invention. These examples are not meant to

15   be limiting in any way. The constructs for insertion into a transposon-based vector are provided in a cloning vector labeled pTnMCS.

<u>pTnMCS (base vector)</u>

Bp 1 – 130 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp1-130

20   Bp 133 – 1777 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems) bp2 29-1873

Bp 1783 – 2991 Transposase, from Tn10 (GenBank accession #J01829) bp 108-1316

Bp 2992 – 3344 Non coding DNA from vector pNK2859

Bp 3345 – 3387 Lambda DNA from pNK2859

25   Bp 3388 – 3457 70 bp of IS10 left from Tn10

Bp 3464 – 3670 Multiple cloning site from pBluescriptII sk(-), thru the XmaI site bp924-718

Bp 3671 - 3715 Multiple cloning site from pBluescriptII sk(-), from the XmaI site thru the XhoI site. These base pairs are usually lost when cloning into pTnMCS.bp

30   717-673

Bp 3716 – 4153 Multiple cloning site from pBluescriptII sk(-), from the XhoI site bp672-235

Bp 4159 - 4228 70 bp of IS10 left from Tn10

Bp 4229 - 4270 Lambda DNA from pNK2859

85

Bp 4271 – 5114 Non-coding DNA from pNK2859

Bp 5115 - 7315 pBluescript sk (-) base vector (Stratagene, Inc.) bp 761-2961

pTnMCS (CMV-prepro-ent-hGH-CPA)

5   Bp 1 – 3670 from vector PTnMCS, bp 1 - 3670

Bp 3676 – 5320 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy
Systems), bp 230-1864

Bp 5326 - 5496 Capsite/Prepro taken from GenBank accession # X07404, bp 563 –
733

10   Bp 5504 - 5652 Synthetic spacer sequence and hairpin loop of HIV gp41 with an
added enterokinase cleavage site

Bp 5653 – 6306 Human growth hormone taken from GenBank accession # V00519,
bp 1-654

Bp 6313 – 6720 Conalbumin polyA taken from GenBank accession # Y00407, bp
15   10651-11058

Bp 6722 –10321 from cloning vector pTnMCS, bp 3716-7315

pTnMCS (CMV-CHOVg-ent-ProInsulin-synPA) (SEQ ID NO:41)

Bp 1 – 3670 from vector PTnMCS, bp 1 - 3670

20   Bp 3676 – 5320 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy
Systems), bp 230-1864

Bp 5327 –6480 Chicken ovalbumin gene taken from GenBank accession # V00383,
bp 66-1219

Bp 6487 - 6636 Synthetic spacer sequence and hairpin loop of HIV gp41 with an
25   added enterokinase cleavage site

Bp 6637 – 6897 Human Proinsulin taken from GenBank accession # NM000207, bp
117-377

Bp 6898 – 6942 Spacer DNA, derived as an artifact from the cloning vectors pTOPO
Blunt II (Invitrogen) and pGWIZ (Gene Therapy Systems)

30   Bp 6943 - 7295 Synthetic polyA from the cloning vector pGWIZ (Gene Therapy
Systems), bp 1920-2271

Bp 7296 – 10895 from cloning vector pTnMCS, bp 3716-7315

pTnMCS (CMV-prepro-ent-ProInsulin-synPA)

Bp 1 – 3670 from vector PTnMCS, bp 1 - 3670

Bp 3676 – 5320 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems), bp 230-1864

5   Bp 5326 - 5496 Capsite/Prepro taken from GenBank accession # X07404, bp 563 – 733

Bp 5504 - 5652 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 5653 – 5913 Human Proinsulin taken from GenBank accession # NM000207, bp

10   117-377

Bp 5914 – 5958 Spacer DNA, derived as an artifact from the cloning vectors pTOPO Blunt II (Invitrogen) and pGWIZ (Gene Therapy Systems)

Bp 5959 - 6310 Synthetic polyA from the cloning vector pGWIZ (Gene Therapy Systems), bp 1920-2271

15   Bp 6313 – 9912 from cloning vector pTnMCS, bp 3716-7315

pTnMCS(Chicken OVep+OVg'+ENT+proins+syn polyA)

Bp 1 – 3670 from vector pTnMCS, bp 1 - 3670

Bp 3676 – 4350 Chicken Ovalbumin enhancer taken from GenBank accession

20   #S82527.1 bp 1-675

Bp 4357 – 5692 Chicken Ovalbumin promoter taken from GenBank accession # J00895M24999 bp 1-1336

Bp 5699 – 6917 Chicken Ovalbumin gene from GenBank Accession # V00383.1 bp 2-1220. (This sequence includes the 5'UTR, containing putative cap site, bp 5699-

25   5762.)

Bp 6924 - 7073 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 7074 - 7334 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 7335 - 7379 Spacer DNA, derived as an artifact from the cloning vectors pTOPO

30   Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 7380 - 7731 Synthetic polyA from the cloning vector gWIZ (Gene Therapy Systems) bp 1920 - 2271

Bp 7733 – 11332 from vector pTnMCS, bp 3716 - 7315

ATLLIB02 133492.1

pTnMCS(Chicken OVep+prepro+ENT+proins+syn polyA)

Bp 1 – 3670 from cloning vector pTnMCS, bp 1 - 3670

Bp 3676 – 4350 Chicken Ovalbumin enhancer taken from GenBank accession #
S82527.1 bp 1-675

Bp 4357 – 5692 Chicken Ovalbumin promoter taken from GenBank accession #
J00895-M24999 bp 1-1336

Bp 5699 – 5869 Cecropin cap site and Prepro, Genbank accession # X07404 bp 563-
733

Bp 5876 - 6025 Synthetic spacer sequence and hairpin loop of HIV gp41 with an
added enterokinase cleavage site

Bp 6026 - 6286 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 6287 - 6331 Spacer DNA, derived as an artifact from the cloning vectors pTOPO
Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 6332 - 6683 Synthetic polyA from the cloning vector gWIZ (Gene Therapy
Systems) bp 1920 - 2271

Bp 6685 – 10284 from cloning vector pTnMCS, bp 3716 - 7315

pTnMCS(Quail OVep+OVg'+ENT+proins+syn polyA)

Bp 1 – 3670 from cloning vector pTnMCS, bp 1 - 3670

Bp 3676 – 4333 Quail Ovalbumin enhancer: 658 bp sequence, amplified in-house
from quail genomic DNA, roughly equivalent to the far-upstream chicken ovalbumin
enhancer, GenBank accession # S82527.1, bp 1-675. (There are multiple base pair
substitutions and deletions in the quail sequence, relative tochicken, so the number of
bases does not correspond exactly.)

Bp 4340 – 5705 Quail Ovalbumin promoter: 1366 bp sequence, amplified in-house
from quail genomic DNA, roughly corresponding to chicken ovalbumin promoter,
GenBank accession # J00895-M24999 bp 1-1336. (There are multiple base pair
substitutions and deletions between the quail and chicken sequences, so the number of
bases does not correspond exactly.)

Bp 5712 – 6910 Quail Ovalbumin gene, EMBL accession # X53964, bp 1-1199. (This
sequence includes the 5'UTR, containing putative cap site bp 5712-5764.)

Bp 6917 - 7066 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 7067 - 7327 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 7328 - 7372 Spacer DNA, derived as an artifact from the cloning vectors pTOPO

5   Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 7373 - 7724 Synthetic polyA from the cloning vector gWIZ (Gene Therapy Systems) bp 1920 - 2271

Bp 7726 – 11325 from cloning vector pTnMCS, bp 3716 - 7315

10   <u>pTnMCS (CHOVep-prepro-ent-hGH-CPA)</u>

Bp 1 – 3670 from vector PTnMCS, bp 1-3670

Bp 3676 – 4350 Chicken Ovalbumin enhancer taken from GenBank accession # S82527.1, bp 1–675

Bp 4357 - 5692 Chicken Ovalbumin promoter taken from GenBank accession #

15   J00899-M24999, bp 1-1336

Bp 5699 – 5869 Capsite/Prepro taken from GenBank accession # X07404, bp 563–733

Bp 5877 - 6025 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 6026 – 6679 Human growth hormone taken from GenBank accession # V00519,

20   bp 1-654

Bp 6686 – 7093 Conalbumin polyA taken from GenBank accession # Y00407, bp 10651-11058

Bp 7095 – 10694 from cloning vector pTnMCS, bp 3716-7315

25   <u>pTnMCS(Quail OVep+prepro+ENT+proins+syn polyA)</u>

Bp 1 – 3670 from cloning vector pTnMCS, bp 1 - 3670

Bp 3676 – 4333 Quail Ovalbumin enhancer: 658 bp sequence, amplified in-house from quail genomic DNA, roughly equivalent to the far- upstream chicken ovalbumin enhancer, GenBank accession #S82527.1, bp 1-675. (There are multiple base pair

30   substitutions and deletions in the quail sequence, relative to chicken, so the number of bases does not correspond exactly.)

Bp 4340 – 5705 Quail Ovalbumin promoter: 1366 bp sequence, amplified in-house from quail genomic DNA, roughly corresponding to chicken ovalbumin promoter,

GenBank accession # J00895-M24999 bp 1-1336. (There are multiple base pair substitutions and deletions between the quail and chicken sequences, so the number of bases does not correspond exactly.)

Bp 5712 – 5882 Cecropin cap site and Prepro, Genbank accession # X07404 bp 563-733

Bp 5889 - 6038 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 6039 - 6299 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 6300 - 6344 Spacer DNA, derived as an artifact from the cloning vectors pTOPO Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 6345 - 6696 Synthetic polyA from the cloning vector gWIZ (Gene Therapy Systems) bp 1920 - 2271

Bp 6698 – 10297 from cloning vector pTnMCS, bp 3716 - 7315

PTnMOD

Bp 1 - 130 remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp1-130

Bp 133 - 1777 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems) bp229-1873

Bp 1783 - 2991 Transposase, modified from Tn10 (GenBank accession #J01829) bp 108-1316

Bp 2992 - 2994 Engineered stop codon

Bp 2996 - 3411 Synthetic polyA from gWIZ (Gene Therapy Systems) bp 1922 - 2337

Bp 3412 - 3719 Non-coding DNA from vector pNK2859

Bp 3720 - 3762 Lambda DNA from pNK2859

Bp 3763 - 3832 70 bp of IS10 left from Tn10

Bp 3839 – 4045 Multiple cloning site from pBluescriptII sk(-), thru the XmaI site bp 924-718

Bp 4046 - 4090 Multiple cloning site from pBluescriptII sk(-), from the XmaI site thru the XhoI site. These base pairs are usually lost when cloning   into pTnMCS. bp 717-673

Bp 4091 – 4528 Multiple cloning site from pBluescriptII sk(-), from the XhoI site bp 672-235

90

Bp 4534 - 4603 70 bp of IS10 left from Tn10

Bp 4604 - 4645 Lambda DNA from pNK2859

Bp 4646 - 5489 Non-coding DNA from pNK2859

Bp 5490 - 7690 pBluescript sk (-) base vector (Stratagene, INC) bp 761-2961

5

pTnMOD (CHOVep-prepro-ent-hGH-CPA)

Bp 1 – 4045 from vector PTnMCS, bp 1 - 4045

Bp 4051 – 4725 Chicken Ovalbumin enhancer taken from GenBank accession # S82527.1, bp 1 – 675

10   Bp 4732 - 6067 Chicken Ovalbumin promoter taken from GenBank accession # J00899-M24999, bp 1-1336

Bp 6074 – 6245 Capsite/Prepro taken from GenBank accession # X07404, bp 563 – 733

Bp 6252 – 6400 Synthetic spacer sequence and hairpin loop of HIV gp41 with an

15   added enterokinase cleavage site

Bp 6401 – 7054 Human growth hormone taken from GenBank accession # V00519, bp 1-654

Bp 7061 – 7468 Conalbumin polyA taken from GenBank accession # Y00407, bp 10651-11058

20   Bp 7470 – 11069 from cloning vector pTnMCS, bp 3716-7315

pTnMOD (CMV-CHOVg-ent-ProInsulin-synPA) (SEQ ID NO:42)

Bp 1 – 4045 from vector PTnMCS, bp 1 - 4045

Bp 4051 – 5695 CMV promoter/enhancer taken from vector pGWIZ (Gene therapy

25   systems), bp 230-1864

Bp 5702 –6855 Chicken ovalbumin gene taken from GenBank accession # V00383, bp 66-1219

Bp 6862 - 7011 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

30   Bp 7012 – 7272 Human Proinsulin taken from GenBank accession # NM000207, bp 117-377

Bp 7273 – 7317 Spacer DNA, derived as an artifact from the cloning vectors pTOPO Blunt II (Invitrogen) and pGWIZ (Gene Therapy Systems)

91

ATLLIB02 133492.1

Bp 7318 - 7670 Synthetic polyA from the cloning vector pGWIZ (Gene Therapy Systems), bp 1920-2271

Bp 7672 –11271 from cloning vector pTnMCS, bp 3716-7315

5   pTnMOD (CMV-prepro-ent-hGH-CPA)

Bp 1 – 4045 from vector PTnMCS, bp 1 - 4045

Bp 4051 – 5695 CMV promoter/enhancer taken from vector pGWIZ (Gene therapy systems), bp 230-1864

Bp 5701 - 5871 Capsite/Prepro taken fron GenBank accession # X07404, bp 563 –

10   733

Bp 5879 - 6027 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 6028 – 6681 Human growth hormone taken from GenBank accession # V00519, bp 1-654

15   Bp 6688 – 7095 Conalbumin polyA taken from GenBank accession # Y00407, bp 10651-11058

Bp 7097 – 10696 from cloning vector pTnMCS, bp 3716-7315

pTnMOD (CMV-prepro-ent-ProInsulin-synPA)

20   Bp 1 – 4045 from vector PTnMCS, bp 1 - 4045

Bp 4051 – 5695 CMV promoter/enhancer taken from vector pGWIZ (Gene therapy systems), bp 230-1864

Bp 5701 - 5871 Capsite/Prepro taken from GenBank accession # X07404, bp 563 – 733

25   Bp 5879 - 6027 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 6028 – 6288 Human Proinsulin taken from GenBank accession # NM000207, bp 117-377

Bp 6289 – 6333 Spacer DNA, derived as an artifact from the cloning vectors pTOPO

30   Blunt II (Invitrogen) and pGWIZ (Gene Therapy Systems)

Bp 6334 - 6685 Synthetic polyA from the cloning vector pGWIZ (Gene Therapy Systems), bp 1920-2271

Bp 6687 –10286 from cloning vector pTnMCS, bp 3716-7315

pTnMOD(Chicken OVep+OVg'+ENT+proins+syn polyA) (SEQ ID NO:43)

Bp 1 – 4045 from cloning vector pTnMOD, bp 1 - 4045

Bp 4051 – 4725 Chicken Ovalbumin enhancer taken from GenBank accession #
5   S82527.1 bp 1-675

Bp 4732 – 6067 Chicken Ovalbumin promoter taken from GenBank accession #
J00895-M24999 bp 1-1336

Bp 6074 – 7292 Chicken Ovalbumin gene from GenBank Accession # V00383.1 bp
2-1220. (This sequence includes the 5'UTR, containing putative cap site bp 6074-
10   6137.)

Bp 7299 - 7448 Synthetic spacer sequence and hairpin loop of HIV gp41 with an
added enterokinase cleavage site

Bp 7449 - 7709 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 7710 - 7754 Spacer DNA, derived as an artifact from the cloning vectors pTOPO
15   Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 7755 - 8106 Synthetic polyA from the cloning vector gWIZ (Gene Therapy
Systems) bp 1920 - 2271

Bp 8108 – 11707 from cloning vector pTnMCS, bp 3716 - 7315

20   pTnMOD(Chicken OVep+prepro+ENT+proins+syn polyA)

Bp 1 – 4045 from cloning vector pTnMCS, bp 1 - 4045

Bp 4051 – 4725 Chicken Ovalbumin enhancer taken from GenBank accession #
S82527.1 bp 1-675

Bp 4732 – 6067 Chicken Ovalbumin promoter taken from GenBank accession #
25   J00895-M24999 bp 1-1336

Bp 6074 – 6244 Cecropin cap site and Prepro, Genbank accession # X07404 bp 563-
733

Bp 6251 - 6400 Synthetic spacer sequence and hairpin loop of HIV gp41 with an
added enterokinase cleavage site

30   Bp 6401 - 6661 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 6662 - 6706 Spacer DNA, derived as an artifact from the cloning vectors pTOPO
Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

93

Bp 6707 - 7058 Synthetic polyA from the cloning vector gWIZ (Gene Therapy Systems) bp 1920 - 2271

Bp 7060 – 10659 from cloning vector pTnMCS, bp 3716 - 7315

5　pTnMOD(Quail OVep+OVg'+ENT+proins+syn polyA)

Bp 1 – 4045 from cloning vector pTnMCS, bp 1 - 4045

Bp 4051 – 4708 Quail Ovalbumin enhancer: 658 bp sequence, amplified in-house from quail genomic DNA, roughly equivalent to the far-upstream chicken ovalbumin enhancer, GenBank accession # S82527.1, bp 1-675. (There are multiple base pair

10　substitutions and deletions in the quail sequence, relative to chicken, so the number of bases does not correspond exactly.)

Bp 4715 – 6080 Quail Ovalbumin promoter: 1366 bp sequence, amplified in-house from quail genomic DNA, roughly corresponding to chicken ovalbumin promoter, GenBank accession # J00895-M24999 bp 1-1336. (There are multiple base pair

15　substitutions and deletions between the quail and chicken sequences, so the number of bases does not correspond exactly.)

Bp 6087 – 7285 Quail Ovalbumin gene, EMBL accession # X53964, bp 1-1199. (This sequence includes the 5'UTR, containing putative cap site bp 6087-6139.)

Bp 7292 - 7441 Synthetic spacer sequence and hairpin loop of HIV gp41 with an

20　added enterokinase cleavage site

Bp 7442 - 7702 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 7703 - 7747 Spacer DNA, derived as an artifact from the cloning vectors pTOPO Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

Bp 7748 - 8099 Synthetic polyA from the cloning vector gWIZ (Gene Therapy

25　Systems) bp 1920 - 2271

Bp 8101 – 11700 from cloning vector pTnMCS, bp 3716 - 7315

pTnMOD(Quail OVep+prepro+ENT+proins+syn polyA)

Bp 1 – 4045 from cloning vector pTnMCS, bp 1 - 4045

30　Bp 4051 – 4708 Quail Ovalbumin enhancer: 658 bp sequence, amplified in-housefrom quail genomic DNA, roughly equivalent to the far-upstream chicken ovalbumin enhancer, GenBank accession #S82527.1, bp 1-675. (There are multiple

base pair substitutions and deletions in the quail sequence, relative to chicken, so the number of bases does not correspond exactly.)

Bp 4715 – 6080 Quail Ovalbumin promoter: 1366 bp sequence, amplified in-house from quail genomic DNA, roughly corresponding to chicken ovalbumin promoter,

5　GenBank accession # J00895-M24999 bp 1-1336. (There are multiple base pair substitutions and deletions between the quail and chicken sequences, so the number of bases does not correspond exactly.)

Bp 6087 – 6257 Cecropin cap site and Prepro, Genbank accession # X07404 bp 563-733

10　Bp 6264 - 6413 Synthetic spacer sequence and hairpin loop of HIV gp41 with an added enterokinase cleavage site

Bp 6414 - 6674 Human proinsulin GenBank Accession # NM000207 bp 117-377

Bp 6675 - 6719 Spacer DNA, derived as an artifact from the cloning vectors pTOPO Blunt II (Invitrogen) and gWIZ (Gene Therapy Systems)

15　Bp 6720 - 7071 Synthetic polyA from the cloning vector gWIZ (Gene Therapy Systems) bp 1920 - 2271

Bp 7073 – 10672 from cloning vector pTnMCS, bp 3716 - 7315

PTnMod(CMV/Transposase/ChickOvep/prepro/ProteinA/ConpolyA)

20　BP 1-130 remainder of F1 (-) ori of pBluescriptII sk(-) (Stragagene) bp 1-130.

BP 133-1777 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems) bp 229-1873.

BP 1780-2987 Transposase, modified from Tn10 (GenBank #J01829).

BP 2988-2990 Engineered stop codon.

25　BP 2991-3343 non coding DNA from vector pNK2859.

BP 3344-3386 Lambda DNA from pNK2859.

BP 3387-3456 70bp of IS10 left from Tn10.

BP 3457-3674 multiple cloning site from pBluescriptII sk(-) bp 924-707.

BP 3675-5691 Chicken Ovalbumin enhancer plus promoter from a Topo Clone 10

30　maxi 040303 (5' XmaI, 3' BamHI)

BP 5698-5865 prepro with Cap site amplified from cecropin of pMON200 GenBank # X07404 (5'BamHI, 3'KpnI)

ATL.LIB02 1334921

BP 5872-7338 Protein A gene from GenBank# J01786, mature peptide bp 292-1755 (5'KpnI, 3'SacII)

BP 7345-7752 ConPolyA from Chicken conalbumin polyA from GenBank # Y00407 bp 10651-11058. (5'SacII, 3'XhoI)

5   BP 7753-8195 multiple cloning site from pBluescriptII sk(-) bp 677-235.

BP 8196-8265 70 bp of IS10 left from Tn10.

BP 8266-8307 Lamda DNA from pNK2859

BP 8308-9151 noncoding DNA from pNK2859

BP 9152-11352 pBluescriptII sk(-) base vector (Stratagene, INC.) bp 761-2961

10

All patents, publications and abstracts cited above are incorporated herein by reference in their entirety. It should be understood that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the

15   present invention as defined in the following claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1.   A vector comprising:

a)   a modified transposase gene operably-linked to a first promoter; wherein the nucleic acid sequence 3' to the first promoter comprises the sequence as set forth in SEQ ID NO:13, wherein SEQ ID NO:13 contains the Kozak sequence and a start codon for the transposase, and wherein at least one of the first twenty codons for the transposase gene are modified from the wild-type sequence by changing a nucleotide at a third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon, and

b)   one or more genes of interest operably-linked to one or more additional promoters; and wherein the one or more genes of interest and their operably-linked promoters are flanked by transposase insertion sequences recognized by the transposase encoded by the modified transposase gene.

2.   The vector of claim 1, wherein the modified transposase gene comprises an adenine or thymine at the third position in each of codons 2-10 of the modified transposase gene.

3.   The vector of claim 1 or 2, comprising the sequence set forth in SEQ ID NO: 1.

4.   The vector of any one of claims 1 to 3, wherein the transposase is a Tn10 transposase.

5.   The vector of any one of claims 1 to 4, wherein one gene of interest is operably-linked to a second promoter.

6.   The vector according to any one of claims 1 to 5, wherein the first promoter and the second promoter are independently selected from the group consisting of a constitutive promoter and an inducible promoter.

7.   The vector of claim 6, wherein the inducible promoter is selected from the group consisting of an ovalbumin promoter, a conalbumin promoter, a vitellogenin promoter or an ovomucoid promoter.

8.   The vector according to any preceding claim, further comprising a polyA sequence operably- linked to the transposase gene.

9.   The vector of claim 8, wherein the polyA sequence is a conalbumin polyA sequence.

10.   The vector according to any preceding claim, further comprising two stop codons operably-linked to the transposase gene.

11.   The vector of any one of claims 1 to 4, wherein a first gene of interest is operably-linked to a second promoter and a second gene of interest is operably-linked to a third promoter.

12.   The vector of any one of claims 1 to 4, wherein a first and a second gene of interest are operably-linked to a second promoter.

13.   The vector according to any preceding claim, further comprising an enhancer operably-linked to the one or more genes of interest.

14.   The vector of claim 13, wherein the enhancer comprises at least a portion of an ovalbumin enhancer.

15.   The vector according to any preceding claim, further comprising an egg directing sequence operably-linked to the one or more genes of interest.

16.   The vector of claim 15, wherein the egg directing sequence is an ovalbumin signal sequence, an ovomucoid signal sequence or a vitellogenin targeting sequence.

17.   A method of producing a transgenic animal comprising administering to the animal a vector according to any one of claims 1 to 16.

18.   The method of claim 17, wherein the vector is administered via an intratesticular, intraarterial, intraoviductal or intraembryonic route.

19.   The method of claim 17 or 18, wherein the animal is an avian animal.

20.   The method of claim 19, wherein the avian animal is a chicken or a quail.

21.   An egg produced by the transgenic avian animal of claim 19 or 20, wherein the egg contains one or more desired proteins encoded by the one or more genes of interest.

22.   A transgenic sperm produced by the transgenic animal produced according to any one of claims 17 to 20.

23.   A method for producing a desired protein comprising:
        a)   administering to the animal a vector according to any one of claims 1 to 16; and
        b)   isolating the desired protein produced in the animal.

24.   The method of claim 23, wherein the animal is an egg-laying animal, and the method of administration is intraoviductal, such that the desired protein produced by the at least one gene of interest is isolated from the egg white of eggs laid by the egg-laying animal.

25.   The method of claim 23 or 24, wherein the vector further comprises a TAG sequence and wherein the desired protein is purified using the TAG sequence.

26.   The method of claim 25, wherein the TAG sequence comprises: (i) a sequence that encodes a polypeptide that functions as a purification handle; (ii) a cleavage site; and (iii) a polynucleotide spacer.

27.   The method of claim 25 or 26, wherein the TAG sequence comprises a polynucleotide sequence shown in SEQ ID NO: 22.

28.   The method of any one of claims 23 to 25, wherein the desired protein is a lytic protein, proinsulin, or a human growth hormone.

29.   The method according to any one of claims 23 to 28, wherein the vector further comprises a second gene of interest operably-linked to a third promoter and wherein the genes of interest encode antibody polypeptides.

30.   The vector of claim 7, wherein the inducible promoter comprises the sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 40, or nucleic acids 4050-4938 of SEQ ID NO: 30.

31.   The vector of claim 15 or 16, wherein the egg directing sequence comprises at least one of the sequences as set forth in SEQ ID NO: 18, nucleic acids 4960-5112 of SEQ ID NO: 3, nucleic acids 4943-5092 of SEQ ID NO: 4, nucleic acids 4958-6115 of SEQ ID NO: 29, or nucleic acids 4945-6092 of SEQ ID NO: 30.

32.   The vector of claim 8, wherein the polyA sequence comprises at least one of the sequences as set forth in SEQ ID NO: 28, SEQ ID NO: 33, or nucleic acids 2995-3410 of SEQ ID NO: 1.

33.   The vector according to any one of claims 1 to 16, wherein the modified transposase gene comprises an A or a T at the third position in each of codons 2-10 of the modified transposase gene.

34.   The method of claim 23, wherein the vector is administered via an intratesticular, intraarterial, intraperitoneal, intravenous, intraoviductal, intraembryonic, nasal, or pronuclear route.

FIGURE 1

FIGURE 2

FIGURE 3

| Pro | Transposase | Poly A | | IS | Ovo Pro | Ovo SS | TAG | ProInsulin Gene | PolyA | IS |

4/7

FIGURE 4

| Vit Pro | Transposase | Poly A | | IS | Vit Pro | Vit Targ | Proinsulin | Poly A | IS |
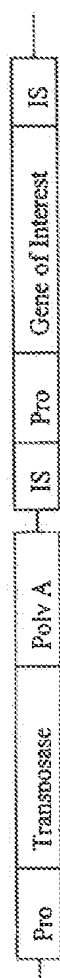
FIGURE 5

IS	Oval Pro	prepro	Heavy chain	pro	Light chain	polyA	IS

FIGURE 6

| IS | Oval Pro | prepro | Light chain | ent | Heavy chain | polyA | IS |

FIGURE 7

A.

Tail-to-Tail

IS	Oval Pro	Oval SS	Light chain	Poly A	Spacer DNA	Poly A	Heavy chain	Oval SS	Oval Pro	IS

B.

Tail-to-Head

IS	Oval Pro	Oval SS	Light chain	Poly A	Spacer DNA	Oval Pro	Oval SS	Heavy chain	Poly A	IS

Appendix A

SEQ ID NO:1 (pTnMod)

```
 5    CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG  50
      CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC  100
      TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG  150
      GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT  200
      CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA  250
10    TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG  300
      CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC  350
      CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA  400
      GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA  450
      CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG  500
15    TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA  550
      TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC  600
      CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG  650
      ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG  700
      TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC  750
20    CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA  800
      GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC  850
      TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG  900
      GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA  950
      CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA  1000
25    CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG  1050
      ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT  1100
      CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT  1150
      GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC  1200
      TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA  1250
30    AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA  1300
      CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG  1350
      CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC  1400
      CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG  1450
      CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG  1500
35    GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG  1550
      CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG  1600
      GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG  1650
      GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC  1700
      CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT  1750
40    CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT  1800
      TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT  1850
      CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA  1900
      CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT  1950
      AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA  2090
45    GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC  2050
      GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC  2100
      TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT  2150
      TATGAGAAAG CGTTCCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA  2200
      CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC  2250
50    TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTGAG  2300
      AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC  2350
      AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT  2400
      CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT  2450
      CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA  2500
55    AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT  2550
      ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT  2600
      GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA  2650
      GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC  2700
      TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA  2750
60    ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA  2800
      GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA  2850
```

103

```
       ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
       TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
       AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
       GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
   5   TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
       CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
       TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
       CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
       GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
  10   CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
       CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
       TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
       CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
       CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
  15   TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
       CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
       TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
       ATCCAAGAGA ACCAACTTAA AGCTTCCTG ACGGAATGTT AATTCTCGTT 3750
       GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
  20   TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
       CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
       TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
       CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
       GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGC 4050
  25   TGCAGGAATT CGATATCAAG CTTATCGATA CCGCTGACCT CGAGGGGGGG 4100
       CCCGGTACCC AATTCGCCCT ATAGTGAGTC GTATTACGCG CGCTCACTGG 4150
       CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT TACCCAACTT 4200
       AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA ATAGCGAAGA 4250
       GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG AATGGCGAAT 4300
  30   GGAAATTGTA AGCGTTAATA TTTTGTTAAA ATTCGCGTTA AATTTTTGTT 4350
       AAATCAGCTC ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT 4400
       AAATCAAAAG AATAGACCGA GATAGGGTTG AGTGTTGTTC CAGTTTGGAA 4450
       CAAGAGTCCA CTATTAAAGA ACGTGGACTC CAACGTCAAA GGGCGAAAAA 4500
       CCGTCTATCA GGGCGATGGC CCACTACTCC GGGATCATAT GACAAGATGT 4550
  35   GTATCCACCT TAACTTAATG ATTTTTACCA AAATCATTAG GGGATTCATC 4600
       AGTGCTCAGG GTCAACGAGA ATTAACATTC CGTCAGGAAA GCTTATGATG 4650
       ATGATGTGCT TAAAAACTTA CTCAATGGCT GGTTATGCAT ATCGCAATAC 4700
       ATGCGAAAAA CCTAAAGAG CTTGCCGATA AAAAAGGCCA ATTTATTGCT 4750
       ATTTACCGCG GCTTTTTATT GAGCTTGAAA GATAAATAAA ATAGATAGGT 4800
  40   TTTATTTGAA GCTAAATCTT CTTTATCGTA AAAAATGCCC TCTTGGGTTA 4850
       TCAAGAGGGT CATTATATTT CGCGGAATAA CATCATTTGG TGACGAAATA 4900
       ACTAAGCACT TGTCTCCTGT TTACTCCCCT GAGCTTGAGG GGTTAACATG 4950
       AAGGTCATCG ATAGCAGGAT AATAATACAG TAAAACGCTA AACCAATAAT 5000
       CCAAATCCAG CCATCCCAAA TTGGTAGTGA ATGATTATAA ATAACAGCAA 5050
  45   ACAGTAATGG GCCAATAACA CCGGTTGCAT TGGTAAGGCT CACCAATAAT 5100
       CCCTGTAAAG CACCTTGCTG ATGACTCTTT GTTTGGATAG ACATCACTCC 5150
       CTGTAATGCA GGTAAAGCGA TCCCACCACC AGCCAATAAA ATTAAAACAG 5200
       GGAAAACTAA CCAACCTTCA GATATAAACG CTAAAAAGGC AAATGCACTA 5250
       CTATCTGCAA TAAATCCGAG CAGTACTGCC GTTTTTTCGC CCATTTAGTG 5300
  50   GCTATTCTTC CTGCCACAAA GGCTTGGAAT ACTGAGTGTA AAAGACCAAG 5350
       ACCCGTAATG AAAAGCCAAC CATCATGCTA TTCATCATCA CGATTTCTGT 5400
       AATAGCACCA CACCGTGCTG GATTGGCTAT CAATGCGCTG AAATAATAAT 5450
       CAACAAATGG CATCGTTAAA TAAGTGATGT ATACCGATCA GCTTTTGTTC 5500
       CCTTTAGTGA GGGTTAATTG CGCGCTTGGC GTAATCATGG TCATAGCTGT 5550
  55   TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACGAGCC 5600
       GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC 5650
       ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT 5700
       GCCAGCTGCA TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCGT 5750
       ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT 5800
  60   TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT 5850
       CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG 5900
```

104

```
    CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG 5950
    GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT 6000
    GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC 6050
    TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC 6100
5   CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA 6150
    GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC 6200
    GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT 6250
    TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG 6300
    GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG 6350
10  AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG 6400
    CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT 6450
    CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG 6500
    CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC 6550
    TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG 6600
15  TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA 6650
    TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG 6700
    TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC 6750
    GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG 6800
    GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG 6850
20  CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG 6900
    AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT 6950
    TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA 7000
    CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGTA 7050
    TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC 7100
25  CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT 7150
    CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC 7200
    ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT 7250
    GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG 7300
    CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT 7350
30  TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG 7400
    ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA 7450
    CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA 7500
    CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT 7550
    TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG 7600
35  TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC 7650
    AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCAC            7689


    SEQ ID NO:2 (PTnMod (CMV/Red))
40

    CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG 50
    CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC 100
    TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG 150
    GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT 200
45  CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA 250
    TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG 300
    CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC 350
    CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA 400
    GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA 450
50  CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG 500
    TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA 550
    TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC 600
    CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG 650
    ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG 700
55  TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC 750
    CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA 800
    GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC 850
    TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG 900
    GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA 950
60  CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA 1000
    CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG 1050
```

```
     ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT 1100
     CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT 1150
     GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC 1200
     TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA 1250
  5  AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA 1300
     CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG 1350
     CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC 1400
     CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG 1450
     CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG 1500
 10  GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG 1550
     CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG 1600
     GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG 1650
     GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC 1700
     CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT 1750
 15  CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT 1800
     TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT 1850
     CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA 1900
     CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT 1950
     AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA 2000
 20  GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC 2050
     GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAA 2100
     TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT 2150
     TATGAGAAAG CGTTCCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA 2200
     CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC 2250
 25  TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTGAG 2300
     AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC 2350
     AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT 2400
     CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT 2450
     CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA 2500
 30  AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT 2550
     ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT 2600
     GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA 2650
     GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC 2700
     TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTGATAT CATGCTGCTA 2750
 35  ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA 2800
     GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA 2850
     ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
     TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
     AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
 40  GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
     TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
     CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
     TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
     CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
 45  GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
     CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
     CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
     TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
     CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
 50  CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
     TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
     CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
     TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
     ATCCAAGAGA ACCAACTTAA AGCTTCCTG ACGGAATGTT AATTCTCGTT 3750
 55  GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
     TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
     CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
     TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
     CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
 60  GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGC 4050
     ATCAGATTGG CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG 4100
```

                                          106

```
      TACATTTATA TTGGCTCATG TCCAACATTA CCGCCATGTT GACATTGATT 4150
      ATTGACTAGT TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC 4200
      CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC 4250
      TGACCGCCCA ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC 4300
 5    CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT 4350
      TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT 4400
      ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC 4450
      CCAGTACATG ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT 4500
      TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG 4550
 10   CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA 4600
      CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT 4650
      GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG 4700
      TGGGAGGTCT ATATAAGCAG AGCTCGTTTA GTGAACCGTC AGATCGCCTG 4750
      GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC CGGGACCGAT 4800
 15   CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT TCCCCGTGCC 4850
      AAGAGTGACG TAAGTACCGC CTATAGACTC TATAGGCACA CCCCTTTGGC 4900
      TCTTATGCAT GCTATACTGT TTTTGGCTTG GGGCCTATAC ACCCCCGCTT 4950
      CCTTATGCTA TAGGTGATGG TATAGCTTAG CCTATAGGTG TGGGTTATTG 5000
      ACCATTATTG ACCACTCCCC TATTGGTGAC GATACTTTCC ATTACTAATC 5050
 20   CATAACATGG CTCTTTGCCA CAACTATCTC TATTGGCTAT ATGCCAATAC 5100
      TCTGTCCTTC AGAGACTGAC ACGGACTCTG TATTTTTACA GGATGGGGTC 5150
      CCATTTATTA TTTACAAATT CACATATACA ACAACGCCGT CCCCCGTGCC 5200
      CGCAGTTTTT ATTAAACATA GCGTGGGATC TCCACGCGAA TCTCGGGTAC 5250
      GTGTTCCGGA CATGGGCTCT TCTCCGGTAG CGGCGGAGCT TCCACATCCG 5300
 25   AGCCCTGGTC CCATGCCTCC AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG 5350
      CTCCTAACAG TGGAGGCCAG ACTTAGGCAC AGCACAATGC CCACCACCAC 5400
      CAGTGTGCCG CACAAGGCCG TGGCGGTAGG GTATGTGTCT GAAAATGAGC 5450
      GTGGAGATTG GGCTCGCACG GCTGACGCAG ATGGAAGACT TAAGGCAGCG 5500
      GCAGAAGAAG ATGCAGGCAG CTGAGTTGTT GTATTCTGAT AAGAGTCAGA 5550
 30   GGTAACTCCC GTTGCGGTGC TGTTAACGGT GGAGGGCAGT GTAGTCTGAG 5600
      CAGTACTCGT TGCTGCCGCG CGCGCCACCA GACATAATAG CTGACAGACT 5650
      AACAGACTGT TCCTTTCCAT GGGTCTTTTC TGCAGTCACC GTCTCGCGAC 5700
      AGGGATCCAC CGGTCGCCAC CATGGTGCGC TCCTCCAAGA ACGTCATCAA 5750
      GGAGTTCATG CGCTTCAAGG TGCGCATGGA GGGCACCGTG AACGGCCACG 5800
 35   AGTTCGAGAT CGAGGGCGAG GGCGAGGGCC GCCCCTACGA GGGCCACAAC 5850
      ACCGTGAAGC TGAAGGTGAC CAAGGGCGGC CCCCTGCCCT TCGCCTGGGA 5900
      CATCCTGTCC CCCCAGTTCC AGTACGGCTC CAAGGTGTAC GTGAAGCACC 5950
      CCGCCGACAT CCCCGACTAC AAGAAGCTGT CCTTCCCCGA GGGCTTCAAG 6000
      TGGGAGCGCG TGATGAACTT CGAGGACGGC GGCGTGGTGA CCGTGACCCA 6050
 40   GGACTCCTCC CTGCAGGACG GCTGCTTCAT CTACAAGGTG AAGTTCATCG 6100
      GCGTGAACTT CCCCTCCGAC GGCCCCGTAA TGCAGAAGAA GACCATGGGC 6150
      TGGGAGGCCT CCACCGAGCG CCTGTACCCC CGCGACGGCG TGCTGAAGGG 6200
      CGAGATCCAC AAGGCCCTGA AGCTGAAGGA CGGCGGCCAC TACCTGGTGG 6250
      AGTTCAAGTC CATCTACATG GCCAAGAAGC CCGTGCAGCT GCCCGGCTAC 6300
 45   TACTACGTGG ACTCCAAGCT GGACATCACC TCCCACAACG AGGACTACAC 6350
      CATCGTGGAG CAGTACGAGC GCACCGAGGG CCGCCACCAC CTGTTCCTGT 6400
      AGCGGCCGCG ACTCTAGATC ATAATCAGCC ATACCACATT TGTAGAGGTT 6450
      TTACTTGCTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA 6500
      AATGAATGCA ATTGTTGTTG TTAACTTGTT TATTGCAGCT TATAATGGTT 6550
 50   ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA 6600
      CTGCATTCTA GTTGTGGCCC GGGCTGCAGG AATTCGATAT CAAGCTTATC 6650
      GATACCGCTG ACCTCGAGGG GGGGCCCGGT ACCCAATTCG CCCTATAGTG 6700
      AGTCGTATTA CGCGCGCTCA CTGGCCGTCG TTTTACAACG TCGTGACTGG 6750
      GAAAACCCTG GCGTTACCCA ACTTAATCGC CTTGCAGCAC ATCCCCCTTT 6800
 55   CGCCAGCTGG CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC 6850
      AGTTGCGCAG CCTGAATGGC GAATGGGAAAT GTAAGCGTT AATATTTTGT 6900
      TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT TAACCAATAG 6950
      GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG 7000
      GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG 7050
 60   ACTCCAACGT CAAAGGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA 7100
      CTCCGGGATC ATATGACAAG ATGTGTATCC ACCTTAACTT AATGATTTTT 7150
```

107

```
        ACCAAAATCA TTAGGGGATT CATCAGTGCT CAGGGTCAAC GAGAATTAAC 7200
        ATTCCGTCAG GAAAGCTTAT GATGATGATG TGCTTAAAAA CTTACTCAAT 7250
        GGCTGGTTAT GCATATCGCA ATACATGCGA AAAACCTAAA AGAGCTTGCC 7300
        GATAAAAAAG GCCAATTTAT TGCTATTTAC CGCGGCTTTT TATTCGAGCTT 7350
   5    GAAAGATAAA TAAAATAGAT AGGTTTTATT TGAAGCTAAA TCTTCTTTAT 7400
        CGTAAAAAAT GCCCTCTTGG GTTATCAAGA GGGTCATTAT ATTTCGCGGA 7450
        ATAACATCAT TTGGTGACGA AATAACTAAG CACTTGTCTC CTGTTTACTC 7500
        CCCTGAGCTT GAGGGGTTAA CATGAAGGTC ATCGATAGCA GGATAATAAT 7550
        ACAGTAAAAC GCTAAACCAA TAATCCAAAT CCAGCCATCC CAAATTGGTA 7600
   10   GTGAATGATT ATAAATAACA GCAAACAGTA ATGGGCCAAT AACACCGGTT 7650
        GCATTGGTAA GGCTCACCAA TAATCCCTGT AAAGCACCTT GCTGATGACT 7700
        CTTTGTTTGG ATAGACATCA CTCCCTGTAA TGCAGGTAAA GCGATCCCAC 7750
        CACCAGCCAA TAAAATTAAA ACAGGGAAAA CTAACCAACC TTCAGATATA 7800
        AACGCTAAAA AGGCAAATGC ACTACTATCT GCAATAAATC CGAGCAGTAC 7850
   15   TGCCGTTTTT TCGCCCATTT AGTGGCTATT CTTCCTGCCA CAAAGGCTTG 7900
        GAATACTGAG TGTAAAAGAC CAAGACCCGT AATGAAAAGC CAACCATCAT 7950
        GCTATTCATC ATCACGATTT CTGTAATAGC ACCACACCGT GCTGGATTGG 8000
        CTATCAATGC GCTGAAATAA TAATCAACAA ATGGCATCGT TAAATAAGTG 8050
        ATGTATACCG ATCAGCTTTT GTTCCCTTTA GTGAGGGTTA ATTGCGCGCT 8100
   20   TGGCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAATTG TTATCCGCTC 8150
        ACAATTCCAC ACAACATACG AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG 8200
        TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCGTTGCGC TCACTGCCCG 8250
        CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA 8300
        CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG CTTCCTCGCT 8350
   25   CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC 8400
        ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA 8450
        AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC 8500
        CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA 8550
        AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA 8600
   30   TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC 8650
        CCTGCCGCTT ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG 8700
        CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT 8750
        CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG 8800
        CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT 8850
   35   TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT 8900
        GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC 8950
        TAGAAGGACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG 9000
        GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC 9050
        GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC 9100
   40   TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG 9150
        AAAACTCACG TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC 9200
        ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT 9250
        ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC 9300
        CTATCTCAGC GATCTGTCTA TTTCGTTCAT CCATAGTTGC CTGACTCCCC 9350
   45   GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC 9400
        TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA 9450
        TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA 9500
        TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG 9550
        TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG 9600
   50   TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA 9650
        CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGGCTACAC 9700
        CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT 9750
        CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC 9800
        GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA 9850
   55   ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA 9900
        ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT 9950
        TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC 10000
        GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA 10050
        CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAAG 10100
   60   GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA 10150
        ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT 10200
```

                                    108

```
TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC 10250
CGAAAAGTGC CAC    10263
```

5   SEQ ID NO:3 (PTnMod (Oval/Red) Chicken)

```
CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG 50
CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC 100
TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG 150
GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT 200
CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA 250
TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG 300
CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC 350
CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA 400
GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA 450
CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG 500
TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA 550
TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC 600
CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG 650
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG 700
TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC 750
CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA 800
GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC 850
TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG 900
GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA 950
CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA 1000
CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG 1050
ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT 1100
CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT 1150
GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC 1200
TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA 1250
AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA 1300
CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG 1350
CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC 1400
CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG 1450
CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG 1500
GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG 1550
CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG 1600
GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG 1650
GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC 1700
CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT 1750
CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT 1800
TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT 1850
CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA 1900
CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT 1950
AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA 2000
GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC 2050
GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC 2100
TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT 2150
TATGAGAAAG CGTTCCCGCT TTCAGAGCAA AAGCTCATGA 2200
CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC 2250
TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTGAG 2300
AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC 2350
AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGGATATGT 2400
CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT 2450
CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA 2500
AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT 2550
ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT 2600
GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA 2650
GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC 2700
TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA 2750
```

109

```
       ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA 2800
       GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA 2850
       ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
       TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
   5   AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
       GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
       TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
       CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
       TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
   10  CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
       GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
       CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
       CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
       TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
   15  CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
       CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
       TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
       CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
       TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
   20  ATCCAAGAGA ACCAACTTAA AGCTTTCCTG ACGGAATGTT AATTCTCGTT 3750
       GACCCTGAAC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
       TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
       CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
       TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
   25  CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
       GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
       AGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG 4100
       AACAATAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG 4150
       TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
   30  ATCTGCCAGG CCATTAAGTT ATTCATGGAA GATCTTTGAG GAACACTGAA 4250
       AGTTCATATC ATAAACACAT TTGAAATTGA GTATTGTTTT GCATTGTATG 4300
       GAGCTATGTT TTGCTGTATC CTCAGAAAAA AAGTTTGTTA TAAAGCATTC 4350
       ACACCCATAA AAAGATAGAT TTAAATATTC CAGCTATAGG AAAGAAAGTG 4400
       CGTCTGCTCT TCACTCTAGT CTCAGTTGGC TCCTTCACAT GCATGCTTCT 4450
   35  TTATTTCTCC TATTTTGTCA AGAAAATAAT AGGTCACGTC TTGTTCTCAC 4500
       TTATGTCCTG CCTAGCATGG CTCAGATGCA CGTTGTAGAT ACAAGAAGGA 4550
       TCAAATGAAA CAGACTTCTG GTCTGTTACT ACAACCATAG TAATAAGCAC 4600
       ACTAACTAAT AATTGCTAAT TATGTTTTCC ATCTCTAAGG TTCCCACATT 4650
       TTTCTGTTTT CTTAAAGATC CCATTATCTG GTTGTAACTG AAGCTCAATG 4700
   40  GAACATGAGC AATATTTCCC AGTCTTCTCT CCCATCCAAC AGTCCTGATG 4750
       GATTAGCAGA ACAGGCAGAA AACACATTGT TACCCAGAAT TAAAAACTAA 4800
       TATTTGCTCT CCATTCAATC CAAAATGGAC CTATTGAAAC TAAAATCTAA 4850
       CCCAATCCCA TTAAATGATT TCTATGGCGT CAAAGGTCAA ACTTCTGAAG 4900
       GGAACCTGTG GGTGGGTCAC AATTCAGGCT ATATATTCCC CAGGGCTCAG 4950
   45  CGGATCTCCA TGGGCTCCAT CGGTGCAGCA AGCATGGAAT TTTGTTTTGA 5000
       TGTATTCAAG GAGCTCAAGA TCCACCATGC CAATGAGAAC ATCTTCTACT 5050
       GCCCCATTGC CATCATGTCA GCTCTAGCCA TGGTATACCT GGGTGCAAAA 5100
       GACAGCACCA GGGAATTCGT GCGCTCCTCC AAGAACGTCA TCAAGGAGTT 5150
       CATGCGCTTC AAGGTGCGCA TGGAGGGCAC CGTGAACGGC CACGAGTTCG 5200
   50  AGATCGAGGG CGAGGGCGAG GGCCGCCCCT ACGAGGGCCA CAACACCGTG 5250
       AAGCTGAAGG TGACCAAGGG CGGCCCCCTG CCCTTCGCCT GGGACATCCT 5300
       GTCCCCCCAG TTCCAGTACG GCTCCAAGGT GTACGTGAAG CACCCCGCCG 5350
       ACATCCCCGA CTACAAGAAG CTGTCCTTCC CCGAGGGCTT CAAGTGGGAG 5400
       CGCGTGATGA ACTTCGAGGA CGGCGGCGTG GTGACCGTGA CCCAGGACTC 5450
   55  CTCCCTGCAG GACGGCTGCT TCATCTACAA GGTGAAGTTC ATCGGCGTGA 5500
       ACTTCCCCTC CGACGGCCCC GTAATGCAGA AGAAGACCAT GGGCTGGGAG 5550
       GCCTCCACCG AGCGCCTGTA CCCCCGCGAC GGCGTGCTGA AGGGCGAGAT 5600
       CCACAAGGCC CTGAAGCTGA AGGACGGCGG CCACTACCTG GTGGAGTTCA 5650
       AGTCCATCTA CATGGCCAAG AAGCCCGTGC AGCTGCCCGG CTACTACTAC 5700
   60  GTGGACTCCA AGCTGGACAT CACCTCCCAC AACGAGGACT ACACCATCGT 5750
       GGAGCAGTAC GAGCGCACCG AGGGCCGCCA CCACCTGTTC CTGTAGCGGC 5800
```

<div align="center">110</div>

```
      CGCGACTCTA GATCATAATC AGCCATACCA CATTTGTAGA GGTTTTACTT 5850
      GCTTTAAAAA ACCTCCCACA CCTCCCCCTG AACCTGAAAC ATAAAATGAA 5900
      TGCAATTGTT GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT 5950
 5    AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT 6000
      TCTAGTTGTG GCTCGAGAAG GGCGAATTCT GCAGATATCC ATCACACTGG 6050
      CGGCCGCTCG AGGGGGGGGCC CGGTACCCAA TTCGCCCTAT AGTGAGTCGT 6100
      ATTACGCGCG CTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC 6150
      CCTGGCGTTA CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG 6200
      CTGGCGTAAT AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC 6250
 10   GCAGCCTGAA TGGCGAATGG AAATTGTAAG CGTTAATATT TTGTTAAAAT 6300
      TCGCGTTAAA TTTTTGTTAA ATCAGCTCAT TTTTTAACCA ATAGGCCGAA 6350
      ATCGGCAAAA TCCCTTATAA ATCAAAAGAA TAGACCGAGA TAGGGTTGAG 6400
      TGTTGTTCCA GTTTGGAACA AGAGTCCACT ATTAAAGAAC GTGGACTCCA 6450
      ACGTCAAAGG GCGAAAAACC GTCTATCAGG GCGATGGCCC ACTACTCCGG 6500
 15   GATCATATGA CAAGATGTGT ATCCACCTTA ACTTAATGAT TTTTACCAAA 6550
      ATCATTAGGG GATTCATCAG TGCTCAACGG GAATAAGGGAA TAACATTCCG 6600
      TCAGGAAAGC TTATGATGAT GATGTGCTTA AAAACTTACT CAATGGCTGG 6650
      TTATGCATAT CGCAATACAT GCGAAAAACC TAAAAGAGCT TGCCGATAAA 6700
      AAAGGCCAAT TTATTGCTAT TTACCGCGGC TTTTTATTGA GCTTGAAAGA 6750
 20   TAAATAAAAT AGATAGGTTT TATTTGAAGC TAAATCTTCT TTATCGTAAA 6800
      AAATGCCCTC TTGGGTTATC AAGAGGGTCA TTATATTTCG CGGAATAACA 6850
      TCATTTGGTG ACGAAATAAC TAAGCACTTG TCTCCTGTTT ACTCCCCTGA 6900
      GCTTGAGGGG TTAACATGAA GGTCATCGAT AGCAGGATAA TAATACAGTA 6950
      AAACGCTAAA CCAATAATCC AAATCCAAAT ATCCCAAATT GGTAGTGAAT 7000
 25   GATTATAAAT AACAGCAAAC AGTAATGGGC CAATAACACC GGTTGCATTG 7050
      GTAAGGCTCA CCAATAATCC CTGTAAAGCA CCTTGCTGAT GACTCTTTGT 7100
      TTGGATAGAC ATCACTCCCT GTAATGCAGG TAAAGCGATC CCACCACCAG 7150
      CCAATAAAAT TAAAACAGGG AAAACTAACC AACCTTCAGA TATAAACGCT 7200
      AAAAAGGCAA ATGCACTACT ATCTGCAATA AATCCGAGCA GTACTGCCGT 7250
 30   TTTTTCGCCC CATTTAGTGG CTATTCTTCC TGCCACAAAG GCTTGGAATA 7300
      CTGAGTGTAA AAGACCAAGA CCCGCTAATG AAAAGCCAAC CATCATGCTA 7350
      TTCCATCCAA AACGATTTTC GGTAAATAGC ACCCACACCG TTGCGGGAAT 7400
      TTGGCCTATC AATTGCGCTG AAAAATAAAT AATCAACAAA ATGGCATCGT 7450
      TTTAAATAAA GTGATGTATA CCGAATTCAG CTTTTGTTCC CTTTAGTGAG 7500
 35   GGTTAATTGC GCGCTTGGCG TAATCATGGT CATAGCTGTT TCCTGTGTGA 7550
      AATTGTTATC CGCTCACAAT TCCACACAAC ATACGAGCCG GAAGCATAAA 7600
      GTGTAAAGCC TGGGGTGCCT AATGAGTGAG CTAACTCACA TTAATTGCGT 7650
      TGCGCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG CCAGCTGCAT 7700
      TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC 7750
 40   TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC 7800
      GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA 7850
      GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG 7900
      GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC 7950
      CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG 8000
 45   ACAGGACTAT AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG 8050
      CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC 8100
      CTTCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT 8150
      TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT 8200
      TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC 8250
 50   CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT 8300
      AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC 8350
      TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA 8400
      AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA 8450
      ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG 8500
 55   CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG 8550
      ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA 8600
      TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA 8650
      ATCAATCTAA AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT 8700
      TAATCAGTGA GGCACCTATC TCAGCGATCT GTCTATTTCG TTCATCCATA 8750
 60   GTTGCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC 8800
      ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC 8850
```

ATLLIB02 1335492.1

```
          CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT 8900
          GGTCCTGCAA CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA 8950
          AGCTAGAGTA AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTTGCCA 9000
          TTGCTACAGG CATCGTGGTG TCACGCTCGT CGTTTGGTAT GGCTTCATTC 9050
5         AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG 9100
          CAAAAAAGCG GTTAGCTCCT TCGGTCCTCC GATCGTTGTC AGAAGTAAGT 9150
          TGGCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT 9200
          ACTGTCATGC CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC 9250
          CAAGTCATTC TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG 9300
10        CGTCAATACG GGATAATACC GCGCCACATA GCAGAACTTT AAAAGTGCTC 9350
          ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT 9400
          GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG 9450
          CATCTTTTAC TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA 9500
          AATGCCGCAA AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT 9550
15        ACTCTTCCTT TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA 9600
          TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA AATAGGGGTT 9650
          CCGCGCACAT TTCCCCGAAA AGTGCCAC    9678
```

20   SEQ ID NO:4 (PTnMod (Oval/Red) Quail)

```
          CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG 50
          CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC 100
          TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG 150
25        GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT 200
          CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA 250
          TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG 300
          CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC 350
          CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA 400
30        GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA 450
          CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG 500
          TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA 550
          TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC 600
          CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG 650
35        ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG 700
          TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC 750
          CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA 800
          GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC 850
          TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG 900
40        GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA 950
          CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA 1000
          CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG 1050
          ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT 1100
          CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT 1150
45        GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC 1200
          TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA 1250
          AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA 1300
          CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG 1350
          CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC 1400
50        CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG 1450
          CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCGAAAG GCCGCACAAG 1500
          GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG 1550
          CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG 1600
          GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG 1650
55        GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC 1700
          CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT 1750
          CCATGGGTCT TTTCTGCAGT CACCGTCCGA CCATGTGTGA ACTTGATATT 1800
          TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT 1850
          CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA 1900
60        CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT 1950
          AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA 2000
```

                                           112

```
        GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC 2050
        GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC 2100
        TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT 2150
        TATGAGAAAG CGTTCCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA 2200
  5     CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC 2250
        TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTCAG 2300
        AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC 2350
        AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT 2400
        CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT 2450
  10    CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA 2500
        AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT 2550
        ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT 2600
        GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA 2650
        GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC 2700
  15    TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA 2750
        ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA 2800
        GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA 2850
        ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
        TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
  20    AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
        GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
        TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
        CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
        TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
  25    CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
        GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
        CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
        CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
        TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
  30    CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
        CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
        TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
        CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
        TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAAATTG 3700
  35    ATCCAAGAGA ACCAACTTAA AGCTTCCTG ACGGAATGTT AATTCTCGTT 3750
        GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
        TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
        CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
        TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
  40    CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
        GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
        AGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG 4100
        AACAAAAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG 4150
        TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
  45    ATCTGCCAGG CTGGAAGATC ATGGAAGATC TCTGAGGAAC ATTGCAAGTT 4250
        CATACCATAA ACTCATTTGG AATTGAGTAT TATTTTGCTT TGAATGGAGC 4300
        TATGTTTTGC AGTTCCCTCA GAAGAAAAGC TTGTTATAAA GCGTCTACAC 4350
        CCATCAAAAG ATATATTTAA ATATTCCAAC TACAGAAAGA TTTTGTCTGC 4400
        TCTTCACTCT GATCTCAGTT GGTTTCTTCA CGTACATGCT TCTTTATTTG 4450
  50    CCTATTTTGT CAAGAAAATA ATAGGTCAAG TCCTGTTCTC ACTTATCTCC 4500
        TGCCTAGCAT GGCTTAGATG CACGTTGTAC ATTCAAGAAG GATCAAATGA 4550
        AACAGACTTC TGGTCTGTTA CAACAACCAT AGTAATAAAC AGACTAACTA 4600
        ATAATTGCTA ATTATGTTTT CCATCTCTAA GGTTCCCACA TTTTTCTGTT 4650
        TTAAGATCCC ATTATCTGGT TGTAACTGAA GCTCAATGGA ACATGAACAG 4700
  55    TATTTCTCAG TCTTTTCTCC AGCAATCCTG ACGGATTAGA AGAACTGGCA 4750
        GAAAACACTT TGTTACCCAG AATTAAAAAC TAATATTTGC TCTCCCTTCA 4800
        ATCCAAAATG GACCTATTGA AACTAAAATC TGACCCAATC CCATTAAATT 4850
        ATTCTATGG CGTCAAAGGT CAAACTTTTG AAGGGAACCT GTGGGTGGGT 4900
        CCCAATTCAG GCTATATATT CCCCAGGGCT CAGCGGATCT CCATGGGCTC 4950
  60    CTCGTGCAGC AAGCATGGAA TTTTGCCTTG ATGTATTCAA GGAGCTCAAA 5000
        GTCCACCATG CCAATGACAA CATGCTCTAC TCCCCCTTTG CCATCTGTCA 5050
```

                                          113

```
ACTCTGGCCA TGGTCTCCCT GGGTGCAAAA GACAGCACCA GGGAATTCGT 5100
GCGCTCCTCC AAGAACGTCA TCAAGGAGTT CATGCGCTTC AAGGTGCGCA 5150
TGGAGGGCAC CGTGAACGGC CACGAGTTCG AGATCGAGGG CGAGGGCGAG 5200
GGCCGCCCCT ACGAGGGCCA CAACACCGTG AAGCTGAAGG TGACCAAGGG 5250
CGGCCCCCTG CCCTTCGCCT GGGACATCCT GTCCCCCCAG TTCCAGTACG 5300
GCTCCAAGGT GTACGTGAAG CACCCCGCCG ACATCCCCGA CTACAAGAAG 5350
CTGTCCTTCC CCGAGGGCTT CAAGTGGGAG CGCGTGATGA ACTTCGAGGA 5400
CGGCGGCGTG GTGACCGTGA CCCAGGACTC CTCCCTGCAG GACGGCTGCT 5450
TCATCTACAA GGTGAAGTTC ATCGGCGTGA ACTTCCCCTC CGACGGCCCC 5500
GTAATGCAGA AGAAGACCAT GGGCTGGGAG GCCTCCACCG AGCGCCTGTA 5550
CCCCCGCGAC GGCGTGCTGA AGGGCGAGAT CCACAAGGCC CTGAAGCTGA 5600
AGGACGGCGG CCACTACCTG GTGGAGTTCA AGTCCATCTA CATGGCCAAG 5650
AAGCCCGTGC AGCTGCCCGG CTACTACTAC GTGGACTCCA AGCTGGACAT 5700
CACCTCCCAC AACGAGGACT ACACCATCGT GGAGCAGTAC GAGCGCACCG 5750
AGGGCCGCCA CCACCTGTTC CTGTAGCGGC CGCGACTCTA GATCATAATC 5800
AGCCATACCA CATTTGTAGA GGTTTTACTT GCTTTAAAAA ACCTCCCACA 5850
CCTCCCCCTG AACCTGAAAC ATAAAATGAA TGCAATTGTT GTTGTTAACT 5900
TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT 5950
TTCACAAATA AAGCATTTTT TCACTGCAT TCTAGTTGTG GCTCGAGAAG 6000
GGCGAATTCT GCAGATATCC ATCACACTGG CGGCCGCTCG AGGGGGGGCC 6050
CGGTACCCAA TTCGCCCTAT AGTGAGTCGT ATTACGCGCG CTCACTGGCC 6100
GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA 6150
TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG 6200
CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG 6250
AAATTGTAAG CGTTAATATT TTGTTAAAAT TCGCGTTAAA TTTTTGTTAA 6300
ATCAGCTCAT TTTTTAACCA ATAGGCCGAA ATCGGCAAAA TCCCTTATAA 6350
ATCAAAAGAA TAGACCGAGA TAGGGTTGAG TGTTGTTCCA GTTTGGAACA 6400
AGAGTCCACT ATTAAAGAAC GTGGACTCCA ACGTCAAAGG GCGAAAAACC 6450
GTCTATCAGG GCGATGGCCC ACTACTCCGG GATCATATGA CAAGATGTGT 6500
ATCCACCTTA ACTTAATGAT TTTTACCAAA ATCATTAGGG GATTCATCAG 6550
TGCTCAGGGT CAACGAGAAT TAACATTCCG TCAGGAAAGC TTATGATGAT 6600
GATGTGCTTA AAAACTTACT CAATGGCTGG TTATGCATAT CGCAATACAT 6650
GCGAAAAACC TAAAAGAGCT TGCCGATAAA AAAGGCCAAT TTATTGCTAT 6700
TTACCGCGGC TTTTTATTGA GCTTGAAAGA TAAATAAAAT AGATAGGTTT 6750
TATTTGAAGC TAAATCTTCT TTATCGTAAA AAATGCCCTC TTGGGTTATC 6800
AAGAGGGTCA TTATATTTCG CGGAATAACA TCATTTGGTG ACGAAATAAC 6850
TAAGCACTTG TCTCCTGTTT ACTCCCCTGA GCTTGAGGGG TTAACATGAA 6900
GGTCATCGAT AGCAGGATAA TAATACAGTA AAACGCTAAA CCAATAATCC 6950
AAATCCAGCC ATCCCAAATT GGTAGTGAAT GATTATAAAT AACAGCAAAC 7000
AGTAATGGGC CAATAACACC GGTTGCATTG GTAAGGCTCA CCAATAATCC 7050
CTGTAAAGCA CCTTGCTGAT GACTCTTTGT TTGGATAGAC ATCACTCCCT 7100
GTAATGCAGG TAAAGCGATC CCACCACCAG CCAATAAAAT TAAAACAGGG 7150
AAAACTAACC AACCTTCAGA TATAAACGCT AAAAAGGCAA ATGCACTACT 7200
ATCTGCAATA AATCCGAGCA GTACTGCCGT TTTTTCGCCC CATTTAGTGG 7250
CTATTCTTCC TGCCACAAAG GCTTGGAATA CTGAGTGTAA AAGACCAAGA 7300
CCCGCTAATG AAAAGCCAAC CATCATGCTA TTCCATCCAA AACGATTTTC 7350
GGTAAATAGC ACCCACACCG TTGCGGGAAT TTGGCCTATC AATTGCGCTG 7400
AAAAATAAAT AATCAACAAA ATGGCATCGT TTTAAATAAA GTGATGTATA 7450
CCGAATTCAG CTTTTGTTCC CTTTAGTGAG GGTTAATTGC GCGCTTGGCG 7500
TAATCATGGT CATAGCTGTT TCCTGTGTGA AATTGTTATC CGCTCACAAT 7550
TCCACACAAC ATACGAGCCG GAAGCATAAA GTGTAAAGCC TGGGGTGCCT 7600
AATGAGTGAG CTAACTCACA TTAATTGCGT TGCGCTCACT GCCCGCTTTC 7650
CAGTCGGGAA ACCTGTCGTG CCAGCTGCAT TAATGAATCG GCCAACGCGC 7700
GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG 7750
ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA 7800
AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA 7850
CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT 7900
TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAAT 7950
CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA 8000
GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC 8050
CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT 8100
```

114

```
TCTCATAGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC 8150
CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT 8200
TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG 8250
CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG 8300
CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA 8350
GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA 8400
AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG GTAGCGGTGG 8450
TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG 8500
AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC 8550
TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA 8600
GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG 8650
AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC 8700
TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT 8750
GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA 8800
TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC AGCAATAAAC 8850
CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC 8900
CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC 8950
CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG 9000
TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC 9050
AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT 9100
TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT GTTATCACTC 9150
ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG 9200
ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT 9250
GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC 9300
GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC 9350
GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT 9400
AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC TTTCACCAGC 9450
GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT 9500
AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT 9550
ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA 9600
TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA 9650
AGTGCCAC                                             9658
```

SEQ ID NO:5 (spacer)
(GPGG)_x

SEQ ID NO:6 (spacer)
GPGGPGGGPGG

SEQ ID NO:7 (spacer)
GGGGSGGGGSGGGGS

SEQ ID NO:8 (spacer)
GGGGSGGGGSGGGGSGGGGS

SEQ ID NO:9 (enterokinase cleavage site)
DDDDK

SEQ ID NO:10 (altered transposase Hef forward primer)
ATCTCGAGACCATGTGTGAACTTGATATTTTACATGATTCTCTTTACC

SEQ ID NO:11 (altered transposase Her reverse primer)
GATTGATCATTATCATAATTTCCCCAAAGCGTAACC

115

SEQ ID NO:12 (Xho I restriction site)
CTCGAG

5   SEQ ID NO:13 (modified Kozak sequence)
ACCATG

10   SEQ ID NO:14 (Bcl I restriction site)
TGATCA

SEQ ID NO:15 (CMVf-NgoM IV primer)
TTGCCGGCATCAGATTGGCTAT

15

SEQ ID NO:16 (Syn-polyAr-BstE II primer)
AGAGGTCACCGGGTCAATTCTTCAGCACCTGGTA

20

SEQ ID NO:17 (vitellogenin promoter)

     TGAATGTGTT CTTGTGTTAT CAATATAAAT CACAGTTAGT GATGAAGTTG GCTGCAAGCC
     TGCATCAGTT CAGCTACTTG GCTGCATTTT GTATTTGGTT CTGTAGGAAA TGCAAAAGGT
25   TCTAGGCTGA CCTGCACTTC TATCCCTCTT GCCTTACTGC TGAGAATCTC TGCAGGTTTT
     AATTGTTCAC ATTTTGCTCC CATTTACTTT GGAAGATAAA ATATTTACAG AATGCTTATG
     AAACCTTTGT TCATTTAAAA ATATTCCTGG TCAGCGTGAC CGGAGCTGAA AGAACACATT
     GATCCCGTGA TTTCAATAAA TACATATGTT CCATATATTG TTTCTCAGTA GCCTCTTAAA
     TCATGTGCGT TGGTGCACAT ATGAATACAT GAATAGCAAA GGTTTATCTG GATTACGCTC
30   TGGCCTGCAG GAATGGCCAT AAACCAAAGC TGAGGGAAGA GGGAGAGTAT AGTCAATGTA
     GATTATACTG ATTGCTGATT GGGTTATTAT CAGCTAGATA ACAACTTGGG TCAGGTGCCA
     GGTCAACATA ACCTGGGCAA AACCAGTCTC ATCTGTGGCA GGACCATGTA CCAGCAGCCA
     GCCGTGACCC AATCTAGGAA AGCAAGTAGC ACATCAATTT TAAATTTATT GTAAATGCCG
     TAGTAGAAGT GTTTTACTGT GATACATTGA AACTTCTGGT CAATCAGAAA AAGGTTTTTT
35   ATCAGAGATG CCAAGGTATT ATTTGATTTT CTTTATTCGC CGTGAAGAGA ATTTATGATT
     GCAAAAAGAG GAGTGTTTAC ATAAACTGAT AAAAAACTTG AGGAATTCAG CAGAAAACAG
     CCACGTGTTC CTGAACATTC TTCCATAAAA GTCTCACCAT GCCTGGCAGA GCCCTATTCA
     CCTTCGCT

40

SEQ ID NO:18 (vitellogenin targeting sequence)

     ATGAGGGGGATCATACTGGCATTAGTGCTCACCCTTGTAGGCAGCCAGAAGTTTGACATTGGT

45

SEQ ID NO:19 (p146 protein)
KYKKALKKLAKLL

50   SEQ ID NO:20 (p146 coding sequence)
AAATACAAAAAAGCACTGAAAAAACTGGCAAAACTGCTG

SEQ ID NO:21 (pro-insulin sequence)
55   TTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGC
     TTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGGCGGG
     GGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAA
     CAATGCTGTACCAGCATCTGCTCCCTCTACCAGCTGGAGAACTCTGCAACTAG

60

SEQ ID NO:22 (TAG sequence)

116

Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp

Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Thr Thr Cys Ile Leu Lys Gly Ser Cys

Gly Trp Ile Gly Leu Leu Asp Asp Asp Asp Lys

SEQ ID NO:23 (gp41 epitope)
Ala Thr Thr Cys Ile Leu Lys Gly Ser Cys Gly Trp Ile Gly Leu Leu

SEQ ID NO:24 (polynucleotide sequence encoding gp41 epitope)
Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Thr Thr Cys Ile Leu Lys Gly
Ser Cys Gly Trp Ile Gly Leu Leu Asp Asp Asp Asp Lys

SEQ ID NO:25 (repeat domain in TAG spacer sequence)
Pro Ala Asp Asp Ala

SEQ ID NO:26 (TAG spacer sequence)
Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp
Ala Pro Ala Asp Asp Ala Pro Ala Asp Asp

SEQ ID NO:27 (Vit pro/Vit targ/TAG/pro-insulin/synthetic polyA)
```
TGAATGTGTT CTTGTGTTAT CAATATAAAT CACAGTTAGT GATGAAGTTG GCTGCAAGCC
TGCATCAGTT CAGCTACTTG GCTGCATTTT GTATTTGGTT CTGTAGGAAA TGCAAAAGGT
TCTAGGCTGA CCTGCACTTC TATCCCTCTT GCCTTACTGC TGAGAATCTC TGCAGGTTTT
AATTGTTCAC ATTTTGCTCC CATTTACTTT GGAAGATAAA ATATTTACAG AATGCTTATG
AAACCTTTGT TCATTTAAAA ATATTCCTGG TCAGCGTGAC CGGAGCTGAA AGAACACATT
GATCCCGTGA TTTCAATAAA TACATATGTT CCATATATTG TTTCTCAGTA GCCTCTTAAA
TCATGTGCGT TGGTGCACAT ATGAATACAT GAATAGCAAA GGTTTATCTG GATTACGCTC
TGGCCTGCAG GAATGGCCAT AAACCAAAGC TGAGGGAAGA GGGAGAGTAT AGTCAATGTA
GATTATACTG ATTGCTGATT GGGTTATTAT CAGCTAGATA ACAACTTGGG TCAGGTGCCA
GGTCAACATA ACCTGGGCAA AACCAGTCTC ATCTGTGGCA GGACCATGTA CCAGCAGCCA
GCCGTGACCC AATCTAGGAA AGCAAGTAGC ACATCAATTT TAAATTTATT GTAAATGCCG
TAGTAGAAGT GTTTTACTGT GATACATTGA AACTTCTGGT CAATCAGAAA AAGGTTTTTT
ATCAGAGATG CCAAGGTATT ATTTGATTTT CTTTATTCGC CGTGAAGAGA ATTTATGATT
GCAAAAAGAG GAGTGTTTAC ATAAACTGAT AAAAAACTTG AGGAATTCAG CAGAAAACAG
CCACGTGTTC CTGAACATTC TTCCATAAAA GTCTCACCAT GCCTGGCAGA GCCCTATTCA
CCTTCGCTAT GAGGGGGATC ATACTGGCAT TAGTGCTCAC CCTTGTAGGC AGCCAGAAGT
TTGACATTGG TAGACTGAGA ATGGCAAGAA GAATGAGAAGA TGGTTTGTG      AACCAACACC
TGTGCGGCTCA CACCTGGTGG AAGCTCTCTA CCTAGTGTGCG GGGAACGAGG CTTCTTCTAC
ACACCCAAGA CCCGCCGGGA GGCAGAGGAC CTGCAGGTGGG GCAGGTGGAG CTGGGCGGGG
GCCCTGGTGC AGGCAGCCTG CAGCCCTTGG CCCTGGAGGGG TCCCTGCAGA AGCGTGGCAT
TGTGGAACAA TGCTGTACCA GCATCTGCTC CCTCTACCAGC TGGAGAACTA CTGCAACTAG
GGCGCCTGGATCCAGATCACTTCTGGCTAATAAAAGATCAGAGCTCTAGAGATCTGTGTGTTGGTTTTT
TGTGGATCTGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACC
CTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG
TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGCACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGG
CATGCTGGGGATGCGGTGGGCTCTATGGGTACCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCGGTACCTCTCTC
```

SEQ ID NO:28 (synthetic polyA sequence)
```
GGCGCCTGGATCCAGATCACTTCTGGCTAATAAAAGATCAGAGCTCTAGAGATCTGTGTGTTGGTTTTT
TGTGGATCTGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACC
CTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG
TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGCACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGG
CATGCTGGGGATGCGGTGGGCTCTATGGGTACCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCGGTACCTCTCTC
```
117

SEQ ID NO:29 (pTnMod(Oval/ENT tag/P146/PA) - Chicken)

```
CTGACGCGCC  CTGTAGCGGC  GCATTAAGCG  CGGCGGGTGT  GGTGGTTACG    50
CGCAGCGTGA  CCGCTACACT  TGCCAGCGCC  CTAGCGCCCG  CTCCTTTCGC   100
TTTCTTCCCT  TCCTTTCTCG  CCACGTTCGC  CGGCATCAGA  TTGGCTATTG   150
GCCATTGCAT  ACGTTGTATC  CATATCATAA  TATGTACATT  TATATTGGCT   200
CATGTCCAAC  ATTACCGCCA  TGTTGACATT  GATTATTGAC  TAGTTATTAA   250
TAGTAATCAA  TTACGGGGTC  ATTAGTTCAT  AGCCCATATA  TGGAGTTCCG   300
CGTTACATAA  CTTACGGTAA  ATGGCCCGCC  TGGCTGACCG  CCCAACGACC   350
CCCGCCCATT  GACGTCAATA  ATGACGTATG  TTCCCATAGT  AACGCCAATA   400
GGGACTTTCC  ATTGACGTCA  ATGGGTGGAG  TATTTACGGT  AAACTGCCCA   450
CTTGGCAGTA  CATCAAGTGT  ATCATATGCC  AAGTACGCCC  CCTATTGACG   500
TCAATGACGG  TAAATGGCCC  GCCTGGCATT  ATGCCCAGTA  CATGACCTTA   550
TGGGACTTTC  CTACTTGGCA  GTACATCTAC  GTATTAGTCA  TCGCTATTAC   600
CATGGTGATG  CGGTTTTGGC  AGTACATCAA  TGGGCGTGGA  TAGCGGTTTG   650
ACTCACGGGG  ATTTCCAAGT  CTCCACCCCA  TTGACGTCAA  TGGGAGTTTG   700
TTTTGGCACC  AAAATCAACG  GGACTTTCCA  AAATGTCGTA  ACAACTCCGC   750
CCCATTGACG  CAAATGGGCG  GTAGGCGTGT  ACGGTGGGAG  GTCTATATAA   800
GCAGAGCTCG  TTTAGTGAAC  CGTCAGATCG  CCTGGAGACG  CCATCCACGC   850
TGTTTTGACC  TCCATAGAAG  ACACCGGGAC  CGATCCAGCC  TCCGCGGCCG   900
GGAACGGTGC  ATTGGAACGC  GGATTCCCCG  TGCCAAGAGT  GACGTAAGTA   950
CCGCCTATAG  ACTCTATAGG  CACACCCCTT  TGGCTCTTAT  GCATGCTATA  1000
CTGTTTTTGG  CTTGGGGCCT  ATACACCCCC  GCTTCCTTAT  GCTATAGGTG  1050
ATGGTATAGC  TTAGCCTATA  GGTGTGGGTT  ATTGACCATT  ATTGACCACT  1100
CCCCTATTGG  TGACGATACT  TTCCATTACT  AATCCATAAC  ATGGCTCTTT  1150
GCCACAACTA  TCTCTATTGG  CTATATGCCA  ATACTCTGTC  CTTCAGAGAC  1200
TGACACGGAC  TCTGTATTTT  TACAGGATGG  GGTCCCATTT  ATTATTTACA  1250
AATTCACATA  TACAACAACG  CCGTCCCCCG  TGCCCGCAGT  TTTTATTAAA  1300
CATAGCGTGG  GATCTCCACG  CGAATCTCGG  GTACGTGTTC  CGGACATGGG  1350
CTCTTCTCCG  GTAGCGGCGG  AGCTTCCACA  TCCGAGCCCT  GGTCCCATGC  1400
CTCCAGCGGC  TCATGGTCGC  TCGGCAGCTC  CTTGCTCCTA  ACAGTGGAGG  1450
CCAGACTTAG  GCACAGCACA  ATGCCCACCA  CCACCAGTGT  GCCGCACAAG  1500
GCCGTGGCGG  TAGGGTATGT  GTCTGAAAAT  GAGCGTGGAG  ATTGGGCTCG  1550
CACGGCTGAC  GCAGATGGAA  GACTTAAGGC  ACGGGCAGAA  GAAGATGCAG  1600
GCAGCTGAGT  TGTTGTATTC  TGATAAGAGT  CAGAGGTAAC  TCCCGTTGCG  1650
GTGCTGTTAA  CGGTGGAGGG  CAGTGTAGTC  TGAGCAGTAC  TCGTTGCTGC  1700
CGCGCGCGCC  ACCAGACATA  ATAGCTGACA  GACTAACAGA  CTGTTCCTTT  1750
CCATGGGTCT  TTTCTGCAGT  CACCGTCGGA  CCATGTGTGA  ACTTGATATT  1800
TTACATGATT  CTCTTTACCA  ATTCTGCCCC  GAATTACACT  TAAAACGACT  1850
CAACAGCTTA  ACGTTGGCTT  GCCACGCATT  ACTTGACTGT  AAAACTCTCA  1900
CTCTTACCGA  ACTTGGCCGT  AACCTGCCAA  CCAAAGCGAG  AACAAAACAT  1950
AACATCAAAC  GAATCGACCG  ATTGTTAGGT  AATCGTCACC  TCCACAAAGA  2000
GCGACTCGCT  GTATACCGTT  GGCATGCTAG  CTTTATCTGT  TCGGGAATAC  2050
GATGCCCATT  GTACTTGTTG  ACTGGTCTGA  TATTCGTGAG  CAAAAACGAC  2100
TTATGGTATT  GCGAGCTTCA  GTCGCACTAC  ACGGTCGTTC  TGTTACTCTT  2150
TATGAGAAAG  CGTTCCCGCT  TTCAGAGCAA  TGTTCAAAGA  AAGCTCATGA  2200
CCAATTTCTA  GCCGACCTTG  CGAGCATTCT  ACCGAGTAAC  ACCACACCGC  2250
TCATTGTCAG  TGATGCTGGC  TTTAAAGTGC  CATGGTATAA  ATCCGTTGAG  2300
AAGCTGGGTT  GGTACTGGTT  AAGTCGAGTA  AGAGGAAAAG  TACAATATGC  2350
AGACCTAGGA  GCGGAAAACT  GGAAACCTAT  CAGCAACTTA  CATGATATGT  2400
CATCTAGTCA  CTCAAAGACT  TTAGGCTATA  AGAGGCTGAC  TAAAAGCAAT  2450
CCAATCTCAT  GCCAAATTCT  ATTGTATAAA  TCTCGCTCTA  AAGGCCGAAA  2500
AAATCAGCGC  TCGACACGGA  CTCATTGTCA  CCACCCGTCA  CCTAAAATCT  2550
ACTCAGCGTC  GGCAAAGGAG  CCATGGGTTC  TAGCAACTAA  CTTACCTGTT  2600
GAAATTCGAA  CACCCAAACA  ACTTGTTAAT  ATCTATTCGA  AGCGAATGCA  2650
GATTGAAGAA  ACCTTCCGAG  ACTTGAAAAG  TCCTGCCTAC  GGACTAGGCC  2700
TACGCCAGTA  CCGAACGAGC  AGCTCAGAGC  GTTTTGATAT  CATGCTGCTA  2750
ATCGCCCTGA  TGCTTCAACT  AACATGTTGG  CTTGCGGGCG  TTCATGCTCA  2800
GAAACAAGGT  TGGGACAAGC  ACTTCCAGGC  TAACACAGTC  AGAAATCGAA  2850
ACGTACTCTC  AACAGTTCGC  TTAGGCATGG  AAGTTTTGCG  GCATTCTGGC  2900
```

118

```
     TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
     AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
     GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
     TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
5    CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
     TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
     CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
     GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
     CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
10   CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
     TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
     CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
     CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
     TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
15   CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
     TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
     ATCCAAGAGA ACCAACTTAA AGCTTCCTG ACGGAATGTT AATTCTCGTT 3750
     GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
     TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
20   CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
     TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
     CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
     GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
     AGGTCAGAAT GGTTTCTTTA CTGTTGTCA ATTCTATTAT TTCAATACAG 4100
25   AACAATAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG 4150
     TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
     ATCTGCCAGG CCATTAAGTT ATTCATGGAA GATCTTTGAG GAACACTGCA 4250
     AGTTCATATC ATAAACACAT TTGAAATTGA GTATTGTTTT GCATTGTATG 4300
     GAGCTATGTT TTGCTGTATC CTCAGAAAAA AAGTTTGTTA TAAAGCATTC 4350
30   ACACCCATAA AAAGATAGAT TTAAATATTC CAGCTATAGG AAAGAAAGTG 4400
     CGTCTGCTCT TCACTCTAGT CTCAGTTGGC TCCTTCACAT GCATGCTTCT 4450
     TTATTTCTCC TATTTTGTCA AGAAAATAAT AGGTCACGTC TTGTTCTCAC 4500
     TTATGTCCTG CCTAGCATGG CTCAGATGCA CGTTGTAGAT ACAAGAAGGA 4550
     TCAAATGAAA CAGACTTCTG GTCTGTTACT ACAACCATAG TAATAAGCAC 4600
35   ACTAACTAAT AATTGCTAAT TATGTTTTCC ATCTCTAAGG TTCCCACATT 4650
     TTTCTGTTTT CTTAAAGATC CCATTATCTG GTTGTAACTG AAGCTCAATG 4700
     GAACATGAGC AATATTTCCC AGTCTTCTCT CCCATCCAAC AGTCCTGATG 4750
     GATTAGCAGA ACAGGCAGAA AACACATTGT TACCCAGAAT TAAAAACTAA 4800
     TATTTGCTCT CCATTCAATC CAAAATGGAC CTATTGAAAC TAAAATCTAA 4850
40   CCCAATCCCA TTAAATGATT TCTATGGCGT CAAAGGTCAA ACTTCTGAAG 4900
     GGAACCTGTG GGTGGGTCAC AATTCAGGCT ATATATTCCC CAGGGCTCAG 4950
     CGGATCCATG GGCTCCATCG GCGCAGCAAG CATGGAATTT TGTTTTGATG 5000
     TATTCAAGGA GCTCAAAGTC CACCATGCCA ATGAGAACAT CTTCTACTGC 5050
     CCCATTGCCA TCATGTCAGC TCTAGCCATG GTATACCTGG GTGCAAAAGA 5100
45   CAGCACCAGG ACACAGATAA ATAAGGTTGT TCGCTTTGAT AAACTTCCAG 5150
     GATTCGGAGA CAGTATTGAA GCTCAGTGTG GCACATCTGT AAACGTTCAC 5200
     TCTTCACTTA GAGACATCCT CAACCAAATC ACCAAACCAA ATGATGTTTA 5250
     TTCGTTCAGC CTTGCCAGTA GACTTTATGC TGAAGAGAGA TACCCAATCC 5300
     TGCCAGAATA CTTGCAGTGT GTGAAGGAAC TGTATAGAGG AGGGTTGGAA 5350
50   CCTATCAACT TTCAAACAGC TGCAGATCAA GCCAGAGAGC TCATCAATTC 5400
     CTGGGTAGAA AGTCAGACAA ATGGAATTAT CAGAAATGTC CTTCAGCCAA 5450
     GCTCCGTGGA TTCTCAAACT GCAATGGTTC TGGTTAATGC CATTGTCTTC 5500
     AAAGGACTGT GGGAGAAAAC ATTTAAGGAT GAAGACACAC AACCAATGCC 5550
     TTTCAGAGTG ACTGAGCAAG AAAGCAAACC TGTGCAGATG ATGTACCAGA 5600
55   TTGGTTTATT TAGAGTGGCA TCAATGGCTT CTGAGAAAAT GAAGATCCTG 5650
     GAGCTTCCAT TTGCCAGTGG GACAATGAGC ATGTTGGTGC TGTTGCCTGA 5700
     TGAAGTCTCA GGCCTTGAGC AGCTTGAGAG TATAATCAAC TTTGAAAAAC 5750
     TGACTGAATG GACCAGTTCT AATGTTATGG AAGAGAGGAA GATCAAAGTG 5800
     TACTTACCTC GCATGAAGAT GGAGGAAAAA TACAACCTCA CATCTGTCTT 5850
60   AATGGCTATG GGCATTACTG ACGTGTTTAG CTCTTCAGCC AATCTGTCTG 5900
     GCATCTCCTC AGCAGAGAGC CTGAAGATAT CTCAAGCTGT CCATGCAGCA 5950
```

119

```
        CATGCAGAAA TCAATGAAGC AGGCAGAGAG GTGGTAGGGT CAGCAGAGGC 6000
        TGGAGTGGAT GCTGCAAGCG TCTCTGAAGA ATTTAGGGCT GACCATCCAT 6050
        TCCTCTTCTG TATCAAGCAC ATCGCAACCA ACGCCGTTCT CTTCTTTGGC 6100
        AGATGTGTTT CCCCTCCGCG GCCAGCAGAT GACGCACCAG CAGATGACGC 6150
  5     ACCAGCAGAT GACGCACCAG CAGATGACGC ACCAGCAGAT GACGCACCAG 6200
        CAGATGACGC AACAACATGT ATCCTGAAAG GCTCTTGTGG CTGGATCGGC 6250
        CTGCTGGATG ACGATGACAA AAAATACAAA AAAGCACTGA AAAAACTGGC 6300
        AAAACTGCTG TAATGAGGGC GCCTGGATCC AGATCACTTC TGGCTAATAA 6350
        AAGATCAGAG CTCTAGAGAT CTGTGTGTTG GTTTTTTGTG GATCTGCTGT 6400
  10    GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT 6450
        TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA 6500
        ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT 6550
        GGGGCAGCAC AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG 6600
        GGGATGCGGT GGGCTCTATG GGTACCTCTC TCTCTCTCTC TCTCTCTCTC 6650
  15    TCTCTCTCTC TCTCTCGGTA CCTCTCTCGA GGGGGGGCCC GGTACCCAAT 6700
        TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG TCGTTTTACA 6750
        ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG 6800
        CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT 6850
        CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GGCGAATGGA AATTGTAAGC 6900
  20    GTTAATATTT TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT 6950
        TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT 7000
        AGACCGAGAT AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA 7050
        TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG 7100
        CGATGGCCCA CTACTCCGGG ATCATATGAC AAGATGTGTA TCCACCTTAA 7150
  25    CTTAATGATT TTTACCAAAA TCATTAGGGG ATTCATCAGT GCTCAGGGTC 7200
        AACGAGAATT AACATTCCGT CAGGAAAGCT TATGATGATG ATGTGCTTAA 7250
        AAACTTACTC AATGGCTGGT TATGCATATC GCAATACATG CGAAAAACCT 7300
        AAAAGAGCTT GCCGATAAAA AAGGCCAATT TATTGCTATT TACCGCGGCT 7350
        TTTTATTGAG CTTGAAAGAT AAATAAAATA GATAGGTTTT ATTTGAAGCT 7400
  30    AAATCTTCTT TATCGTAAAA AATGCCCTCT TGGGTTATCA AGAGGGTCAT 7450
        TATATTTCGC GGAATAACAT CATTTGGTGA CGAAATAACT AAGCACTTGT 7500
        CTCCTGTTTA CTCCCCTGAG CTTGAGGGGT TAACATGAAG GTCATCGATA 7550
        GCAGGATAAT AATACAGTAA AACGCTAAAC CAATAATCCA AATCCAGCCA 7600
        TCCCAAATTG GTAGTGAATG ATTATAAATA ACAGCAAACA GTAATGGGCC 7650
  35    AATAACACCG GTTGCATTGG TAAGGCTCAC CAATAATCCC TGTAAAGCAC 7700
        CTTGCTGATG ACTCTTTGTT TGGATAGACA TCACTCCCTG TAATGCAGGT 7750
        AAAGCGATCC CACCACCAGC CAATAAAATT AAAACAGGGA AAACTAACCA 7800
        ACCTTCAGAT ATAAACGCTA AAAAGGCAAA TGCACTACTA TCTGCAATAA 7850
        ATCCGAGCAG TACTGCCGTT TTTTCGCCCC ATTTAGTGGC TATTCTTCCT 7900
  40    GCCACAAAGG CTTGGAATAC TGAGTGTAAA AGACCAAGAC CCGCTAATGA 7950
        AAAGCCAACC ATCATGCTAT TCCATCCAAA ACGATTTTCG GTAAATAGCA 8000
        CCCACACCGT TGCGGGAATT TGGCCTATCA ATTGCGCTGA AAAATAAATA 8050
        ATCAACAAAA TGGCATCGTT TTAAATAAAG TGATGTATAC CGAATTCAGC 8100
        TTTTGTTCCC TTTAGTGAGG GTTAATTGCG CGCTTGGCGT AATCATGGTC 8150
  45    ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA 8200
        TACGAGCCGG AAGCATAAAG TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC 8250
        TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA 8300
        CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG 8350
        GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC 8400
  50    TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT 8450
        ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA 8500
        AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT 8550
        TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG 8600
        TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC 8650
  55    CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA 8700
        TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC 8750
        ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT 8800
        GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC 8850
        TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC 8900
  60    AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG 8950
        AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT 9000
```

                                    120

```
GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG 9050
CTCTTGATCC GGCAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT 9100
GCAAGCAGCA GATTACGCGC AGAAAAAAAG GATCTCAAGA AGATCCTTTG 9150
ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT CACGTTAAGG 9200
GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG ATCCTTTTAA 9250
ATTAAAAATG AAGTTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG 9300
TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG 9350
TCTATTTCGT TCATCCATAG TTGCCTGACT CCCCGTCGTG TAGATAACTA 9400
CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA 9450
GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC AGCCAGCCGG 9500
AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT 9550
CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT 9600
TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC 9650
GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA 9700
CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG 9750
ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC 9800
AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG 9850
TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA 9900
CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG 9950
CAGAACTTTA AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC 10000
TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT 10050
GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG 10100
AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC 10150
GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT 10200
TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA 10250
AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCAC       10297


    SEQ ID NO:30 (pTnMod(Oval/ENT tag/P146/PA) - QUAIL)

CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG   50
CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC  100
TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC  150
GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT  200
CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA  250
TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG  300
CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC  350
CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA  400
GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA  450
CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG  500
TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA  550
TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC  600
CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG  650
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG  700
TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC  750
CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA  800
GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC  850
TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG  900
GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA  950
CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA 1000
CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG 1050
ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT 1100
CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT 1150
GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC 1200
TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA 1250
AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA 1300
CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG 1350
CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC 1400
CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG 1450
CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG 1500
GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG 1550
```

121

```
        CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG 1600
        GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG 1650
        GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC 1700
        CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT 1750
5       CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT 1800
        TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT 1850
        CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA 1900
        CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT 1950
        AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA 2000
10      GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC 2050
        GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC 2100
        TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT 2150
        TATGAGAAAG CGTTCCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA 2200
        CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC 2250
15      TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTGAG 2300
        AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC 2350
        AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT 2400
        CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT 2450
        CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA 2500
20      AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT 2550
        ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT 2600
        GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA 2650
        GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC 2700
        TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA 2750
25      ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA 2800
        GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA 2850
        ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
        TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
        AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
30      GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
        TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
        CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
        TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
        CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
35      GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
        CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
        CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
        TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
        CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
40      CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
        TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
        CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
        TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
        ATCCAAGAGA ACCAACTTAA AGCTTTCCTG ACGGAATGTT AATTCTCGTT 3750
45      GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
        TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
        CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
        TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
        CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
50      GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
        AGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG 4100
        AACAAAAGCT TCTATAACTA AAATATATTT GCTATTGTAT ATTATGATTG 4150
        TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
        ATCTGCCAGG CTGGAAGATC ATGGAAGATC TCTGAGGAAC ATTGCAAGTT 4250
55      CATACCATAA ACTCATTTGG AATTGAGTAT TATTTTGCTT TGAATGGAGC 4300
        TATGTTTTGC AGTTCCCTCA GAAGAAAAGC TTGTTATAAA GCGTCTACAC 4350
        CCATCAAAAG ATATATTTAA ATATTCCAAC TACAGAAAGA TTTTGTCTGC 4400
        TCTTCACTCT GATCTCAGTT GGTTTCTTCA CGTACATGCT TCTTTATTTG 4450
        CCTATTTTGT CAAGAAAATA ATAGGTCAAG TCCTGTTCTC ACTTATCTCC 4500
60      TGCCTAGCAT GGCTTAGATG CACGTTGTAC ATTCAAGAAG GATCAAATGA 4550
        AACAGACTTC TGGTCTGTTA CAACAACCAT AGTAATAAAC AGACTAACTA 4600
                                    122
```

```
ATAATTGCTA ATTATGTTTT CCATCTCTAA GGTTCCCACA TTTTTCTGTT 4650
TTAAGATCCC ATTATCTGGT TGTAACTGAA GCTCAATGGA ACATGAACAG 4700
TATTTCTCAG TCTTTTCTCC AGCAATCCTG ACGGATTAGA AGAACTGGCA 4750
GAAAACACTT TGTTACCCAG AATTAAAAAC TAATATTTGC TCTCCCTTCA 4800
ATCCAAAATG GACCTATTGA AACTAAAATC TGACCCAATC CCATTAAATT 4850
ATTTCTATGG CGTCAAAGGT CAAACTTTTG AAGGGAACCT GTGGGTGGGT 4900
CCCAATTCAG GCTATATATT CCCCAGGGCT CAGCCAGTGG ATCCATGGGC 4950
TCCATCGGTG CAGCAAGCAT GGAATTTTGT TTTGATGTAT TCAAGGAGCT 5000
CAAACTCCAC CATGCCAATG ACAACATGCT CTACTCCCCC TTTGCCATCT 5050
TGTCAACTCT GGCCATGGTC TTCCTAGGTG CAAAAGACAG CACCAGGACC 5100
CAGATAAATA AGGTTGTTCA CTTTGATAAA CTTCCAGGAT TCGGAGACAC 5150
TATTGAAGCT CAGTGTGGCA CATCTGTAAA TGTTCACTCT TCACTTAGAG 5200
ACATACTCAA CCAAATCACC AAACAAAATG ATGCTTATTC GTTCAGCCTT 5250
GCCAGTAGAC TTTATGCTCA AGAGACATAC ACAGTCGTGC CGGAATACTT 5300
GCAATGTGTG AAGGAACTGT ATAGAGGAGG CTTAGAATCC GTCAACTTTC 5350
AAACAGCTGC AGATCAAGCC AGAGGCCTCA TCAATGCCTG GGTAGAAAGT 5400
CAGACAAACG GAATTATCAG AAACATCCTT CAGCCAAGCT CCGTGGATTC 5450
TCAAACTGCA ATGGTCCTGG TTAATGCCAT TGCCTTCAAG GGACTGTGGG 5500
AGAAAGCATT TAAGGCTGAA GACACGCAAA CAATACCTTT CAGAGTGACT 5550
GAGCAAGAAA GCAAACCTGT GCAGATGATG TACCAGATTG GTTCATTTAA 5600
AGTGGCATCA ATGGCTTCTG AGAAAATGAA GATCCTGGAG CTTCCATTTG 5650
CCAGTGGAAC AATGAGCATG TTGGTGCTGT TGCCTGATGA TGTCTCAGGG 5700
CTTGAGCAGC TTGAGAGTAT AATCAGCTTT GAAAAACTGA CTGAATGGAC 5750
CAGTTCTAGT ATTATGGAAG AGAGGAAGGT CAAAGTGTAC TTACCTCGCA 5800
TGAAGATGGA GGAGAAATAC AACCTCACAT CTCTCTTAAT GGCTATGGGA 5850
ATTACTGACC TGTTCAGCTC TTCAGCCAAT CTGTCTGGCA TCTCCTCAGT 5900
AGGGAGCCTG AAGATATCTC AAGCTGTCCA TGCAGCACAT CGCAGAAATCA 5950
ATGAAGCGGG CAGAGATGTG GTAGGCTCAG CAGAGGCTGG AGTGGATGCT 6000
ACTGAAGAAT TTAGGGCTGA CCATCCATTC CTCTTCTGTG TCAAGCACAT 6050
CGAAACCAAC GCCATTCTCC TCTTTGGCAG ATGTGTTTCT CCGCGGCCAG 6100
CAGATGACGC ACCAGCAGAT GACGCACCAG CAGATGACGC ACCAGCAGAT 6150
GACGCACCAG CAGATGACGC ACCAGCAGAT GACGCAACAA CATGTATCCT 6200
GAAAGGCTCT TGTGGCTGGA TCGGCCTGCT GGATGACGAT GACAAAAAAT 6250
ACAAAAAAGC ACTGAAAAAA CTGGCAAAAC TGCTGTAATG AGGGCGCCTG 6300
GATCCAGATC ACTTCTGGCT AATAAAAGAT CAGAGCTCTA GAGATCTGTG 6350
TGTTGGTTTT TTGTGGATCT GCTGTGCCTT CTAGTTGCCA GCCATCTGTT 6400
GTTTGCCCCT CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC 6450
TGTCCTTTCC TAATAAAATG AGGAAATTGC ATCGCATTGT CTGAGTAGGT 6500
GTCATTCTAT TCTGGGGGGT GGGGTGGGGC AGCACAGCAA GGGGGAGGAT 6550
TGGGAAGACA ATAGCAGGCA TGCTGGGGAT GCGGTGGGCT CTATGGGTAC 6600
CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCTCT 6650
CTCGAGGGGG GGCCCCGGTAC CCAATTCGCC CTATAGTGAG TCGTATTACG 6700
CGCGCTCACT GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC 6750
GTTACCCAAC TTAATCGCCT TGCAGCACAT CCCCCTTTCG CCAGCTGGCG 6800
TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG TTGCGCAGCC 6850
TGAATGGCGA ATGGAAATTG TAAGCGTTAA TATTTTGTTA AAATTCGCGT 6900
TAAATTTTTG TTAAATCAGC TCATTTTTTA ACCAATAGGC CGAAATCGGC 6950
AAAATCCCTT ATAAATCAAA AGAATAGACC GAGATAGGGT TGAGTGTTGT 7000
TCCAGTTTGG AACAAGAGTC CACTATTAAA GAACGTGGAC TCCAACGTCA 7050
AAGGGCGAAA AACCGTCTAT CAGGGCGATG GCCCACTACT CCGGGATCAT 7100
ATGACAAGAT GTGTATCCAC CTTAACTTAA TGATTTTTAC CAAAATCATT 7150
AGGGGATTCA TCAGTGCTCA GGGTCAACGA GAATTAACAT TCCGTCAGGA 7200
AAGCTTATGA TGATGATGTG CTTAAAAACT TACTCAATGG CTGGTTATGC 7250
ATATCGCAAT ACATGCGAAA AACCTAAAAG AGCTTGCCGA TAAAAAAGGC 7300
CAATTTATTG CTATTTACCG CGGCTTTTTA TTGAGCTTGA AGATAAATA 7350
AAATAGATAG GTTTTATTTG AAGCTAAATC TTCTTTATCG TAAAAAATGC 7400
CCTCTTGGGT TATCAAGAGG GTCATTATAT TTCGCGGAAT AACATCATTT 7450
GGTGACGAAA TAACTAAGCA CTTGTCTCCT GTTTACTCCC CTGAGCTTGA 7500
GGGGGTTAACA TGAAGGTCAT CGATAGCAGG ATAATAATAC AGTAAAACGC 7550
TAAACCAATA ATCCAAATCC AGCCATCCCA AATTGGTAGT GAATGATTAT 7600
AAATAACAGC AAACAGTAAT GGGCCAATAA CACCGGTTGC ATTGGTAAGG 7650
```

```
CTCACCAATA ATCCCTGTAA AGCACCTTGC TGATGACTCT TTGTTTGGAT 7700
AGACATCACT CCCTGTAATG CAGGTAAAGC GATCCCACCA CCAGCCAATA 7750
AAATTAAAAC AGGGAAAACT AACCAACCTT CAGATATAAA CGCTAAAAAG 7800
GCAAATGCAC TACTATCTGC AATAAATCCG AGCAGTACTG CCGTTTTTTC 7850
GCCCCATTTA GTGGCTATTC TTCCTGCCAC AAAAGGCTTGG AATACTGAGT 7900
GTAAAGACC AAGACCCGCT AATGAAAAGC CAACCATCAT GCTATTCCAT 7950
CCAAAACGAT TTTCGGTAAA TAGCACCCAC ACCGTTGCGG GAATTTGGCC 8000
TATCAATTGC GCTGAAAAAT AAATAATCAA CAAAATGGCA TCGTTTTAAA 8050
TAAAGTGATG TATACCGAAT TCAGCTTTTG TTCCCTTTAG TGAGGGTTAA 8100
TTGCGCGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT 8150
TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA 8200
AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT 8250
CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA 8300
ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC 8350
TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG 8400
TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT 8450
AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG 8500
TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG 8550
AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA 8600
CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC 8650
TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG 8700
GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG 8750
TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC 8800
CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA 8850
GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA 8900
GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA 8950
CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG 9000
TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC 9050
GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA 9100
AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC 9150
AGTGGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA 9200
AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT 9250
CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA 9300
GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC 9350
TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG 9400
CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT 9450
TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT 9500
GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG 9550
AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT GCCATTGCTA 9600
CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC 9650
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA 9700
AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG 9750
CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC 9800
ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC 9850
ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA 9900
TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT 9950
GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG 10000
ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT 10050
TTACTTTCAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC 10100
GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT 10150
CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG 10200
GATACATaTT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC 10250
ACATTTCCCC GAAAAGTGCC AC                               10272
```

SEQ ID NO:31 (pTnMod(Oval/ENT tag/Proins/PA)- Chicken)

```
CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG  50
CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC 100
TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG 150
GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT 200
```

124

```
CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA  250
TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG  300
CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC  350
CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA  400
GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA  450
CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG  500
TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA  550
TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC  600
CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG  650
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG  700
TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC  750
CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA  800
GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC  850
TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG  900
GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA  950
CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA  1000
CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG  1050
ATGGTATAGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT  1100
CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT  1150
GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC  1200
TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA  1250
AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA  1300
CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG  1350
CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC  1400
CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG  1450
CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG  1500
GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG  1550
CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG  1600
GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG  1650
GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC  1700
CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT  1750
CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT  1800
TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT  1850
CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA  1900
CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCCAG AACAAAACAT  1950
AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA  2000
GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC  2050
GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC  2100
TTATGGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT  2150
TATGAGAAAG CGTTCCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA  2200
CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC  2250
TCATTGTCAG TGATGCTGGC TTTAAAGTGC CATGGTATAA ATCCGTTGAG  2300
AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC  2350
AGACCTAGGA GCGGAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT  2400
CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT  2450
CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGGCCGAAA  2500
AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCCGTCA CCTAAAATCT  2550
ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT  2600
GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA  2650
GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC  2700
TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA  2750
ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA  2800
GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA  2850
ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC  2900
TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA  2950
AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA  3000
GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG  3050
TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC CCACTGTCCT  3100
CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT  3150
TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT  3200
CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA  3250
```

```
GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
ATCCAAGAGA ACCAACTTAA AGCTTTCCTG ACGGAATGTT AATTCTCGTT 3750
GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
AGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG 4100
AACAATAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG 4150
TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
ATCTGCCAGG CCATTAAGTT ATTCATGGAA GATCTTTGAG GAACACTGCA 4250
AGTTCATATC ATAAACACAT TTGAAATTGA GTATTGTTTT GCATTGTATG 4300
GAGCTATGTT TTGCTGTATC CTCAGAAAAA AAGTTTGTTA TAAAGCATTC 4350
ACACCCATAA AAAGATAGAT TTAAATATTC CAGCTATAGG AAAGAAAGTG 4400
CGTCTGCTCT TCACTCTAGT CTCAGTTGGC TCCTTCACAT GCATGCTTCT 4450
TTATTTCTCC TATTTTGTCA AGAAAATAAT AGGTCACGTC TTGTTCTCAC 4500
TTATGTCCTG CCTAGCATGG CTCAGATGCA CGTTGTAGAT ACAAGAAGGA 4550
TCAAATGAAA CAGACTTCTG GTCTGTTACT ACAACCATAG TAATAAGCAC 4600
ACTAACTAAT AATTGCTAAT TATGTTTTCC ATCTCTAAGG TTCCCACATT 4650
TTTCTGTTTT CTTAAAGATC CCATTATCTG GTTGTAACTG AAGCTCAATG 4700
GAACATGAGC AATATTTCCC AGTCTTCTCT CCCATCCAAC AGTCCTGATG 4750
GATTAGCAGA ACAGGCAGAA AACACATTGT TACCCAGAAT TAAAAACTAA 4800
TATTTGCTCT CCATTCAATC CAAAATGGAC CTATTGAAAC TAAAATCTAA 4850
CCCAATCCCA TTAAATGATT TCTATGGCGT CAAAGGTCAA ACTTCTGAAG 4900
GGAACCTGTG GGTGGGTCAC AATTCAGGCT ATATATTCCC CAGGGCTCAG 4950
CGGATCCATG GGCTCCATCG GCGCAGCAAG CATGGAATTT TGTTTTGATG 5000
TATTCAAGGA GCTCAAAGTC CACCATGCCA ATGAGAACAT CTTCTACTGC 5050
CCCATTGCCA TCATGTCAGC TCTAGCCATG GTATACCTGG GTGCAAAAGA 5100
CAGCACCAGG ACACAGATAA ATAAGGTTGT TCGCTTTGAT AAACTTCCAG 5150
GATTCGGAGA CAGTATTGAA GCTCAGTGTG GCACATCTGT AAACGTTCAC 5200
TCTTCACTTA GAGACATCCT CAACCAAATC ACCAAACCAA ATGATGTTTA 5250
TTCGTTCAGC CTTGCCAGTA GACTTTATGC TGAAGAGAGA TACCCAATCC 5300
TGCCAGAATA CTTGCAGTGT GTGAAGGAAC TGTATAGAGG AGGCTTGGAA 5350
CCTATCAACT TTCAAACAGC TGCAGATCAA GCCAGAGAGC TCATCAATTC 5400
CTGGGTAGAA AGTCAGACAA ATGGAATTAT CAGAAATGTC CTTCAGCCAA 5450
GCTCCGTGGA TTCTCAAACT GCAATGGTTC TGGTTAATGC CATTGTCTTC 5500
AAAGGACTGT GGGAGAAAAC ATTTAAGGAT GAAGACACAC AAGCAATGCC 5550
TTTCAGAGTG ACTGAGCAAG AAAGCAAACC TGTGCAGATG ATGTACCAGA 5600
TTGGTTTATT TAGAGTGGCA TCAATGGCTT CTGAGAAAAT GAAGATCCTG 5650
GAGCTTCCAT TTGCCAGTGG GACAATGAGC ATGTTGGTGC TGTTGCCTGA 5700
TGAAGTCTCA GGCCTTGAGC AGCTTGAGAG TATAATCAAC TTTGAAAAAC 5750
TGACTGAATG GACCAGTTCT AATGTTATGG AAGAGAGGAA GATCAAAGTG 5800
TACTTACCTC GCATGAAGAT GGAGGAAAAA TACAACCTCA CATCTGTCTT 5850
AATGGCTATG GGCATTACTG ACGTGTTTAG CTCTTCAGCC AATCTGTCTG 5900
GCATCTCCTC AGCAGAGAGC CTGAAGATAT CTCAAGCTGT CCATGCAGCA 5950
CATGCAGAAA TCAATGAAGC AGGCAGAGAG GTGGTAGGGT CAGCAGAGGC 6000
TGGAGTGGAT GCTGCAAGCG TCTCTGAAGA ATTTAGGGCT GACCATCCAT 6050
TCCTCTTCTG TATCAAGCAC ATCGCAACCA ACGCCGTTCT CTTCTTTGGC 6100
AGATGTGTTT CCCCTCCGCG GCCAGCAGAT GACGCACCAG CAGATGACGC 6150
ACCAGCAGAT GACGCACCAG CAGATGACGC ACCAGCAGAT GACGCACCAG 6200
CAGATGACGC AACAACATGT ATCCTGAAAG CTCTTGTGG CTGGATCGGC 6250
CTGCTGGATG ACGATGACAA ATTTGTGAAC CAACACCTGT GCGGCTCACA 6300
```

126

```
CCTGGTGGAA  GCTCTCTACC  TAGTGTGCGG  GGAACGAGGC  TTCTTCTACA  6350
CACCCAAGAC  CCGCCGGGAG  GCAGAGGACC  TGCAGGTGGG  GCAGGTGGAG  6400
CTGGGCGGGG  GCCCTGGTGC  AGGCAGCCTG  CAGCCCTTGG  CCCTGGAGGG  6450
GTCCCTGCAG  AAGCGTGGCA  TTGTGGAACA  ATGCTGTACC  AGCATCTGCT  6500
CCCTCTACCA  GCTGGAGAAC  TACTGCAACT  AGGGCGCCTG  GATCCAGATC  6550
ACTTCTGGCT  AATAAAAGAT  CAGAGCTCTA  GAGATCTGTG  TGTTGGTTTT  6600
TTGTGGATCT  GCTGTGCCTT  CTAGTTGCCA  GCCATCTGTT  GTTTGCCCCT  6650
CCCCCGTGCC  TTCCTTGACC  CTGGAAGGTG  CCACTCCCAC  TGTCCTTTCC  6700
TAATAAAATG  AGGAAATTGC  ATCGCATTGT  CTGAGTAGGT  GTCATTCTAT  6750
TCTGGGGGGT  GGGGTGGGGC  AGCACAGCAA  GGGGGAGGAT  TGGGAAGACA  6800
ATAGCAGGCA  TGCTGGGGAT  GCGGTGGGCT  CTATGGGTAC  CTCTCTCTCT  6850
CTCTCTCTCT  CTCTCTCTCT  CTCTCTCTCT  CGGTACCTCT  CTCGAGGGGG  6900
GGCCCGGTAC  CCAATTCGCC  CTATAGTGAG  TCGTATTACG  CGCGCTCACT  6950
GGCCGTCGTT  TTACAACGTC  GTGACTGGGA  AAACCCTGGC  GTTACCCAAC  7000
TTAATCGCCT  TGCAGCACAT  CCCCCTTTCG  CCAGCTGGCG  TAATAGCGAA  7050
GAGGCCCGCA  CCGATCGCCC  TTCCCAACAG  TTGCGCAGCC  TGAATGGCGA  7100
ATGGAAATTG  TAAGCGTTAA  TATTTTGTTA  AAATTCGCGT  TAAATTTTTG  7150
TTAAATCAGC  TCATTTTTTA  ACCAATAGGC  CGAAATCGGC  AAAATCCCTT  7200
ATAAATCAAA  AGAATAGACC  GAGATAGGGT  TGAGTGTTGT  TCCAGTTTGG  7250
AACAAGAGTC  CACTATTAAA  GAACGTGGAC  TCCAACGTCA  AAGGGCGAAA  7300
AACCGTCTAT  CAGGGCGATG  GCCCACTACT  CCGGGATCAT  ATGACAAGAT  7350
GTGTATCCAC  CTTAACTTAA  TGATTTTTAC  CAAAATCATT  AGGGGATTCA  7400
TCAGTGTCA   GGGTCAACGA  GAATTAACAT  TCCGTCAGGA  AAGCTTATGA  7450
TGATGATGTG  CTTAAAAACT  TACTCAATGG  CTGGTTATGC  ATATCGCAAT  7500
ACATGCGAAA  AACCTAAAAG  AGCTTGCCGA  TAAAAAAGGC  CAATTTATTG  7550
CTATTTACCG  CGGCTTTTTA  TTGAGCTTGA  AAGATAAATA  AAATAGATAG  7600
GTTTTATTTG  AAGCTAAATC  TTCTTTATCG  TAAAAAATGC  CCTCTTGGGT  7650
TATCAAGACG  GTCATTATAT  TTCGCGGAAT  AACATCATTT  GGTGACGAAA  7700
TAACTAAGCA  CTTGTCTCCT  GTTTACTCCC  CTGAGCTTGA  GGGGTTAACA  7750
TGAAGGTCAT  CGATAGCAGG  ATAATAATAC  AGTAAAACGC  TAAACCAATA  7800
ATCCAATCC   AGCCATCCCA  AATTGGTAGT  GAATGATTAT  AAATAACAGC  7850
AAACAGTAAT  GGGCCAATAA  CACCGGTTGC  ATTGGTAAGG  CTCACCAATA  7900
ATCCCTGTAA  AGCACCTTGC  TGATGACTCT  TTGTTTGGAT  AGACATCACT  7950
CCCTGTAATG  CAGGTAAAGC  GATCCCACCA  CCAGCCAATA  AAATTAAAAC  8000
AGGGAAAACT  AACCAACCTT  CAGATATAAA  CGCTAAAAAG  GCAAATGCAC  8050
TACTATCTGC  AATAAATCCG  AGCAGTACTG  CCGTTTTTTC  GCCCCATTTA  8100
GTGGCTATTC  TTCCTGCCAC  AAAGGCTTGG  AATACTGAGT  GTAAAAGACC  8150
AAGACCCGCT  AATGAAAAGC  CAACCATCAT  GCTATTCCAT  CCAAAACGAT  8200
TTTCGGTAAA  TAGCACCCAC  ACCGTTGCGG  GAATTTGGCC  TATCAATTGC  8250
GCTGAAAAAT  AAATAATCAA  CAAAATGGCA  TCGTTTTAAA  TAAAGTGATG  8300
TATACCGAAT  TCAGCTTTTG  TTCCCTTTAG  TGAGGGTTAA  TTGCGCGCTT  8350
GGCGTAATCA  TGGTCATAGC  TGTTTCCTGT  GTGAAATTGT  TATCCGCTCA  8400
CAATTCCACA  CAACATACGA  GCCGGAAGCA  TAAAGTGTAA  AGCCTGGGGT  8450
GCCTAATGAG  TGAGCTAACT  CACATTAATT  GCGTTGCGCT  CACTGCCCGC  8500
TTTCCAGTCG  GGAAACCTGT  CGTGCCAGCT  GCATTAATGA  ATCGGCCAAC  8550
GCGCGGGGAG  AGGCGGTTTG  CGTATTGGGC  GCTCTTCCGC  TTCCTCGCTC  8600
ACTGACTCGC  TGCGCTCGGT  CGTTCGGCTG  CGGCGAGCGG  TATCAGCTCA  8650
CTCAAAGGCG  GTAATACGGT  TATCCACAGA  ATCAGGGGAT  AACGCAGGAA  8700
AGAACATGTG  AGCAAAAGGC  CAGCAAAAGG  CCAGGAACCG  TAAAAAGGCC  8750
GCGTTGCTGG  CGTTTTTCCA  TAGGCTCCGC  CCCCCTGACG  AGCATCACAA  8800
AAATCGACGC  TCAAGTCAGA  GGTGGCGAAA  CCCGACAGGA  CTATAAAGAT  8850
ACCAGGCGTT  TCCCCCTGGA  AGCTCCCTCG  TGCGCTCTCC  TGTTCCGACC  8900
CTGCCGCTTA  CCGGATACCT  GTCCGCCTTT  CTCCCTTCGG  GAAGCGTGGC  8950
GCTTTCTCAT  AGCTCACGCT  GTAGGTATCT  CAGTTCGGTG  TAGGTCGTTC  9000
GCTCCAAGCT  GGGCTGTGTG  CACGAACCCC  CCGTTCAGCC  CGACCGCTGC  9050
GCCTTATCCG  GTAACTATCG  TCTTGAGTCC  AACCCGGTAA  GACACGACTT  9100
ATCGCCACTG  GCAGCAGCCA  CTGGTAACAG  GATTAGCAGA  GCGAGGTATG  9150
TAGGCGGTGC  TACAGAGTTC  TTGAAGTGGT  GGCCTAACTA  CGGCTACACT  9200
AGAAGGACAG  TATTTGGTAT  CTGCGCTCTG  CTGAAGCCAG  TTACCTTCGG  9250
AAAAAGAGTT  GGTAGCTCTT  GATCCGGCAA  ACAAACCACC  GCTGGTAGCG  9300
GTGGTTTTTT  TGTTTGCAAG  CAGCAGATTA  CGCGCAGAAA  AAAAGGATCT  9350
```

                                        127

ATLLIB02 1334921

```
        CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA 9400
        AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA 9450
        CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA 9500
        TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC 9550
    5   TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC TGACTCCCCG 9600
        TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT 9650
        GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT 9700
        AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT 9750
        CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT 9800
   10   TCGCCAGTTA ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT 9850
        GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC 9900
        GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC 9950
        TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC 10000
        ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG 10050
   15   TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA 10100
        TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA 10150
        TACCCCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT 10200
        CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG 10250
        ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC 10300
   20   CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG 10350
        GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA 10400
        TATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT 10450
        TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC ACATTTCCCC 10500
        GAAAAGTGCC AC                                         10512
   25
```

```
        SEQ ID NO:32 (pTnMod(Oval/ENT tag/Proins/PA)- QUAIL)

   30   CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG   50
        CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC  100
        TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCATCAGA TTGGCTATTG  150
        GCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT TATATTGGCT  200
        CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC TAGTTATTAA  250
   35   TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG  300
        CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC  350
        CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA  400
        GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA  450
        CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG  500
   40   TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA  550
        TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC  600
        CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG  650
        ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG  700
        TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC  750
   45   CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA  800
        GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG CCATCCACGC  850
        TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC TCCGCGGCCG  900
        GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT GACGTAAGTA  950
        CCGCCTATAG ACTCTATAGG CACACCCCTT TGGCTCTTAT GCATGCTATA 1000
   50   CTGTTTTTGG CTTGGGGCCT ATACACCCCC GCTTCCTTAT GCTATAGGTG 1050
        ATGGTACGGC TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT 1100
        CCCCTATTGG TGACGATACT TTCCATTACT AATCCATAAC ATGGCTCTTT 1150
        GCCACAACTA TCTCTATTGG CTATATGCCA ATACTCTGTC CTTCAGAGAC 1200
        TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATTT ATTATTTACA 1250
   55   AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA 1300
        CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG 1350
        CTCTTCTCCG GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC 1400
        CTCCAGCGGC TCATGGTCGC TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG 1450
        CCAGACTTAG GCACAGCACA ATGCCCACCA CCACCAGTGT GCCGCACAAG 1500
        GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCGTGGAG ATTGGGCTCG 1550
   60   CACGGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG 1600
        GCAGCTGAGT TGTTGTATTC TGATAAGAGT CAGAGGTAAC TCCCGTTGCG 1650
```

                                    128

```
GTGCTGTTAA CGGTGGAGGG CAGTGTAGTC TGAGCAGTAC TCGTTGCTGC 1700
CGCGCGCGCC ACCAGACATA ATAGCTGACA GACTAACAGA CTGTTCCTTT 1750
CCATGGGTCT TTTCTGCAGT CACCGTCGGA CCATGTGTGA ACTTGATATT 1800
TTACATGATT CTCTTTACCA ATTCTGCCCC GAATTACACT TAAAACGACT 1850
CAACAGCTTA ACGTTGGCTT GCCACGCATT ACTTGACTGT AAAACTCTCA 1900
CTCTTACCGA ACTTGGCCGT AACCTGCCAA CCAAAGCGAG AACAAAACAT 1950
AACATCAAAC GAATCGACCG ATTGTTAGGT AATCGTCACC TCCACAAAGA 2000
GCGACTCGCT GTATACCGTT GGCATGCTAG CTTTATCTGT TCGGGAATAC 2050
GATGCCCATT GTACTTGTTG ACTGGTCTGA TATTCGTGAG CAAAAACGAC 2100
TTATCGTATT GCGAGCTTCA GTCGCACTAC ACGGTCGTTC TGTTACTCTT 2150
TATGAGAAAG CGTTCCGCT TTCAGAGCAA TGTTCAAAGA AAGCTCATGA 2200
CCAATTTCTA GCCGACCTTG CGAGCATTCT ACCGAGTAAC ACCACACCGC 2250
TCATTGTCAG TGATGCTGGC TTTAAAGTGA CATGGTATAA ATCCGTTGAG 2300
AAGCTGGGTT GGTACTGGTT AAGTCGAGTA AGAGGAAAAG TACAATATGC 2350
AGACCTAGGA GCGGAAAAACT GGAAACCTAT CAGCAACTTA CATGATATGT 2400
CATCTAGTCA CTCAAAGACT TTAGGCTATA AGAGGCTGAC TAAAAGCAAT 2450
CCAATCTCAT GCCAAATTCT ATTGTATAAA TCTCGCTCTA AAGGCCGAAA 2500
AAATCAGCGC TCGACACGGA CTCATTGTCA CCACCGTCA CCTAAAATCT 2550
ACTCAGCGTC GGCAAAGGAG CCATGGGTTC TAGCAACTAA CTTACCTGTT 2600
GAAATTCGAA CACCCAAACA ACTTGTTAAT ATCTATTCGA AGCGAATGCA 2650
GATTGAAGAA ACCTTCCGAG ACTTGAAAAG TCCTGCCTAC GGACTAGGCC 2700
TACGCCATAG CCGAACGAGC AGCTCAGAGC GTTTTGATAT CATGCTGCTA 2750
ATCGCCCTGA TGCTTCAACT AACATGTTGG CTTGCGGGCG TTCATGCTCA 2800
GAAACAAGGT TGGGACAAGC ACTTCCAGGC TAACACAGTC AGAAATCGAA 2850
ACGTACTCTC AACAGTTCGC TTAGGCATGG AAGTTTTGCG GCATTCTGGC 2900
TACACAATAA CAAGGGAAGA CTTACTCGTG GCTGCAACCC TACTAGCTCA 2950
AAATTTATTC ACACATGGTT ACGCTTTGGG GAAATTATGA TAATGATCCA 3000
GATCACTTCT GGCTAATAAA AGATCAGAGC TCTAGAGATC TGTGTGTTGG 3050
TTTTTTGTGG ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC 3100
CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT 3150
TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT 3200
CTATTCTGGG GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA 3250
GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCTCTCT 3300
CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCGGTAC CTCTCTCTCT 3350
CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CGGTACCAGG TGCTGAAGAA 3400
TTGACCCGGT GACCAAAGGT GCCTTTTATC ATCACTTTAA AAATAAAAAA 3450
CAATTACTCA GTGCCTGTTA TAAGCAGCAA TTAATTATGA TTGATGCCTA 3500
CATCACAACA AAAACTGATT TAACAAATGG TTGGTCTGCC TTAGAAAGTA 3550
TATTTGAACA TTATCTTGAT TATATTATTG ATAATAATAA AAACCTTATC 3600
CCTATCCAAG AAGTGATGCC TATCATTGGT TGGAATGAAC TTGAAAAAAA 3650
TTAGCCTTGA ATACATTACT GGTAAGGTAA ACGCCATTGT CAGCAAATTG 3700
ATCCAAGAGA ACCAACTTAA AGCTTTCCTG ACGGAATGTT AATTCTCGTT 3750
GACCCTGAGC ACTGATGAAT CCCCTAATGA TTTTGGTAAA AATCATTAAG 3800
TTAAGGTGGA TACACATCTT GTCATATGAT CCCGGTAATG TGAGTTAGCT 3850
CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT 3900
TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC 3950
CATGATTACG CCAAGCGCGC AATTAACCCT CACTAAAGGG AACAAAAGCT 4000
GGAGCTCCAC CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGG 4050
AGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG 4100
AACAAAAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG 4150
TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC 4200
ATCTGCCAGG CTGGAAGATC ATGGAAGATC TCTGAGGAAC ATTGCAAGTT 4250
CATACCATAA ACTCATTTGG AATTGAGTAT TATTTTGCTT TGAATGGAGC 4300
TATGTTTTGC AGTTCCTTCA GAAGAAAAGC TTGTTATAAA GCGTCTACAC 4350
CCATCAAAAG ATATATTTAA ATATTCCAAC TACAGAAAGA TTTTGTCTGC 4400
TCTTCACTCT GATCTCAGTT GGTTCTTCA CGTACATGCT TCTTTATTTG 4450
CCTATTTTGT CAAGAAAATA ATAGGTCAAG TCCTGTTCTC ACTTATCTCC 4500
TGCCTAGCAT GGCTTAGATG CACGTTGTAC ATTCAAGAAG GATCAAATGA 4550
AACAGACTTC TGGTCTGTTA CAACAACCAT AGTAATAAAC AGACTAACTA 4600
ATAATTGCTA ATTATGTTTT CCATCTCTAA GGTTCCCACA TTTTTCTGTT 4650
TTAAGATCCC ATTATCTGGT TGTAACTGAA GCTCAATGGA ACATGAACAG 4700
```

129

```
TATTTCTCAG TCTTTTCTCC AGCAATCCTG ACGGATTAGA AGAACTGGCA 4750
GAAAACACTT TGTTACCCAG AATTAAAAAC TAATATTTGC TCTCCCTTCA 4800
ATCCAAAATG GACCTATTGA AACTAAAATC TGACCCAATC CCATTAAATT 4850
ATTTCTATGG CGTCAAAGGT CAAACTTTTG AAGGGAACCT GTGGGTGGGT 4900
CCCAATTCAG GCTATATATT CCCCAGGGCT CAGCCAGTGG ATCCATGGGC 4950
TCCATCGGTG CAGCAAGCAT GGAATTTTGT TTTGATGTAT TCAAGGAGCT 5000
CAAAGTCCAC CATGCCAATG ACAACATGCT CTACTCCCCC TTTGCCATCT 5050
TGTCAACTCT GGCCATGGTC TTCCTAGGTG CAAAAGACAG CACCAGGACC 5100
CAGATAAATA AGGTTGTTCA CTTTGATAAA CTTCCAGGAT TCGGAGACAG 5150
TATTGAAGCT CAGTGTGGCA CATCTGTAAA TGTTCACTCT TCACTTAGAG 5200
ACATACTCAA CCAAATCACC AAACAAAATG ATGCTTATTC GTTCAGCCTT 5250
GCCAGTAGAC TTTATGCTCA AGAGACATAC ACAGTCGTGC CGGAATACTT 5300
GCAATGTGTG AAGGAACTGT ATAGAGGAGG CTTAGAATCC GTCAACTTTC 5350
AAACAGCTGC AGATCAAGCC AGAGGCCTCA TCAATGCCTG GGTAGAAAGT 5400
CAGACAAACG GAATTATCAG AAACATCCTT CAGCCAAGCT CCGTGGATTC 5450
TCAAACTGCA ATGGTCCTGG TTAATGCCAT TGCCTTCAAG GGACTGTGGG 5500
AGAAAGCATT TAAGGCTGAA GACACGCAAA CAATACCTTT CAGAGTGACT 5550
GAGCAAGAAA GCAAACCTGT GCAGATGATG TACCAGATTG GTTCATTTAA 5600
AGTGGCATCA ATGGCTTCTG AGAAAATGAA GATCCTGGAG CTTCCATTTG 5650
CCAGTGGAAC AATGAGCATG TTGGTGCTGT TGCCTGATGA TGTCTCAGGC 5700
CTTGAGCAGC TTGAGAGTAT AATCAGCTTT GAAAAACTGA CTGAATGGAC 5750
CAGTTCTAGT ATTATGGAAG AGAGGAAGGT CAAAGTGTAC TTACCTCGCA 5800
TGAAGATGGA GGAGAAATAC AACCTCACAT CTCTCTTAAT GGCTATGGGA 5850
ATTACTGACC TGTTCAGCTC TTCAGCCAAT CTGTCTGGCA TCTCCTCAGT 5900
AGGGAGCCTG AAGATATCTC AAGCTGTCCA TGCAGCACAT GCAGAAATCA 5950
ATGAAGCGGG CAGAGATGTG GTAGGCTCAG CAGAGGCTGG AGTGGATGCT 6000
ACTGAAGAAT TTAGGGCTGA CCATCCATTC CTCTTCTGTG TCAAGCACAT 6050
CGAAACCAAC GCCATTCTCC TCTTTGGCAG ATGTGTTTCT CCGCGGCCAG 6100
CAGATGACGC ACCAGCAGAT GACGCACCAG CAGATGACGC ACCAGCAGAT 6150
GACGCACCAG CAGATGACGC ACCAGCAGAT GACGCAACAA CATGTATCCT 6200
GAAAGGCTCT TGTGGCTGGA TCGGCCTGCT GGATGACGAT GACAAATTTG 6250
TGAACCAACA CCTGTGCGGC TCACACCTGG TGGAAGCTCT CTACCTAGTG 6300
TGCGGGGAAC GAGGCTTCTT CTACACACCC AAGACCCGCC GGGAGGCAGA 6350
GGACCTGCAG GTGGGGCAGG TGGAGCTGGG CGGGGGGCCCT GGTGCAGGCA 6400
GCCTGCAGCC CTTGGCCCTG GAGGGGTCCC TGCAGAAGCG TGGCATTGTG 6450
GAACAATGCT GTACCAGCAT CTGCTCCCTC TACCAGCTGG AGAACTACTG 6500
CAACTAGCGC GCCTGGATCC AGATCACTTC TGGCTAATAA AAGATCAGAG 6550
CTCTAGAGAT CTGTGTGTTG GTTTTTTGTG GATCTGCTGT GCCTTCTAGT 6600
TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA 6650
AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC 6700
ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGCAC 6750
AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT 6800
GGGCTCTATG GGTACCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC 6850
TCTCTCGGTA CCTCTCTCGA GGGGGGGCCC GGTACCCAAT CGCCCTATA 6900
GTGAGTCGTA TTACGCGCGC TCACTGGCCG TCGTTTTACA ACGTCGTGAC 6950
TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG CACATCCCCC 7000
TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC 7050
AACAGTTGCG CAGCCTGAAT GGCGAATGGA AATTGTAAGC GTTAATATTT 7100
TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA 7150
TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT 7200
AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG 7250
TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA 7300
CTACTCCGGG ATCATATGAC AAGATGTGTA TCCACCTTAA CTTAATGATT 7350
TTTACCAAAA TCATTAGGGG ATTCATCAGT GCTCAGGGTC AACGAGAATT 7400
AACATTCCGT CAGGAAAGCT TATGATGATG ATGTGCTTAA AAACTTACTC 7450
AATGGCTGGT TATGCATATC GCAATACATG CGAAAAACCT AAAAGAGCTT 7500
GCCGATAAAA AAGGCCAATT TATTGCTATT TACCGCGGCT TTTTATTGAG 7550
CTTGAAAGAT AAATAAAATA GATAGGTTTT ATTTGAAGCT AAATCTTCTT 7600
TATCGTAAAA AATGCCCTCT TGGGTTATCA AGAGGGTCAT TATATTTCGC 7650
GGAATAACAT CATTTGGTGA CGAAATAACT AAGCACTTGT CTCCTGTTTA 7700
CTCCCCTGAG CTTGAGGGGT TAACATGAAG GTCATCGATA GCAGGATAAT 7750
```

                                        130

```
AATACAGTAA AACGCTAAAC CAATAATCCA AATCCAGCCA TCCCAAATTG 7800
GTAGTGAATG ATTATAAATA ACAGCAAACA GTAATGGGCC AATAACACCG 7850
GTTGCATTGG TAAGGCTCAC CAATAATCCC TGTAAAGCAC CTTGCTGATG 7900
ACTCTTTGTT TGGATAGACA TCACTCCCTG TAATGCAGGT AAAGCGATCC 7950
CACCACCAGC CAATAAAATT AAAACAGGGA AAACTAACCA ACCTTCAGAT 8000
ATAAACGCTA AAAAGGCAAA TGCACTACTA TCTGCAATAA ATCCGAGCAG 8050
TACTGCCGTT TTTTCGCCCC ATTTAGTCGG TATTCTTCCT GCCACAAAGG 8100
CTTGGAATAC TGAGTGTAAA AGACCAAGAC CCGCTAATGA AAAGCCAACC 8150
ATCATGCTAT TCCATCCAAA ACGATTTTCG GTAAATAGCA CCCACACCGT 8200
TGCCGGAATT TGGCCTATCA ATTGCGCTGA AAAATAAATA ATCAACAAAA 8250
TGGCATCGTT TTAAATAAAG TGATGTATAC CGAATTCAGC TTTTGTTCCC 8300
TTTAGTGAGG GTTAATTGCG CGCTTGGCGT AATCATGGTC ATAGCTGTTT 8350
CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA TACGAGCCGG 8400
AAGCATAAAG TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC TAACTCACAT 8450
TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA CCTGTCGTGC 8500
CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG GTTTGCGTAT 8550
TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC TCGGTCGTTC 8600
GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT ACGGTTATCC 8650
ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA 8700
AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT TTCCATAGGC 8750
TCCGCCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG TCAGAGGTGG 8800
CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC CTGGAAGCTC 8850
CCTCCTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA TACCTGTCCG 8900
CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC ACGCTGTAGG 8950
TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT GTGTGCACGA 9000
ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC TATCGTCTTG 9050
AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC AGCCACTGGT 9100
AACAGGATTA GCAGAGCCAG GTATGTAGGC GGTGCTACAG AGTTCTTGAA 9150
GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT GGTATCTGCG 9200
CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC 9250
GGCAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA 9300
GATTACGCGC AGAAAAAAAG GATCTCAAGA AGATCCTTTG ATCTTTTCTA 9350
CGGGGTCTGA CGCTCAGTGG AACGAAAACT CACGTTAAGG GATTTTGGTC 9400
ATGAGATTAT CAAAAAGGAT CTTCACCTAG ATCCTTTTAA ATTAAAAATG 9450
AAGTTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG TCTGACAGTT 9500
ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCGT 9550
TCATCCATAG TTGCCTGACT CCCCGTCGTG TAGATAACTA CGATACGGGA 9600
GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA GACCCACGCT 9650
CACCGGCTCC AGATTTATCA GCAATAAACC AGCCAGCCGG AAGGGCCGAG 9700
CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT CTATTAATTG 9750
TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT TTGCGCAACG 9800
TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTTGGTATG 9850
GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA CATGATCCCC 9900
CATGTTGTGC AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG ATCGTTGTCA 9950
GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC AGCACTGCAT 10000
AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG TGACTGGTGA 10050
GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA CCGAGTTGCT 10100
CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA 10150
AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT 10200
CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT GCACCCAACT 10250
GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG AGCAAAAACA 10300
GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC GGAAATGTTG 10350
AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT TATCAGGGTT 10400
ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA 10450
ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCAC                10487


SEQ ID NO:33 (conalbumin polyA)
tctgccattg ctgcttcctc tgcccttcct cgtcactctg aatgtggctt cttcgctact
gccacagcaa gaaataaaat ctcaacatct aaatgggttt cctgaggttt ttcaagagtc
gttaagcaca ttccttcccc agcacccctt gctgcaggcc agtgccaggc accaacttgg
```

131

ctactgctgc ccatgagaga aatccagttc aatattttcc aaagcaaaat ggattacata
tgccctagat cctgattaac aggcgtttgt attatctagt gctttcgctt cacccagatt
atcccattgc ctccc

SEQ ID NO:34 (exemplary antibody light chain sequence)

```
  1 gagctcgtga tgacccagac tccatcctcc ctgtctgcct ctctgggaga cagagtcacc
 61 atcagttgca gggcaaatca ggacattagc aattatttaa actggtatca gcagaaacca
121 gatggaactg ttaaactcct gatctactac acatcaagat tacactcagg ggtcccatca
181 aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcaa
241 gaagattttg ccacttactt ttgccaacag ggtaatacgc ttccgtggac gttcggtgga
301 ggcaccaacc tggaaatcaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
361 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
421 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
481 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
541 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
601 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttaa
```

SEQ ID NO:35 (exemplary antibody heavy chain sequence)

```
  1 ctcgagtcag gacctggcct ggtggcgccc tcacagaacc tgtccatcac ttgcactgtc
 61 tctgggtttt cattaaccag ctatggtgta cactgggttc gccagcctcc aggaaagggt
121 ctggaatggc tgggagtaat atggactggt agaagcacaa cttataattc ggctctcatg
181 tccagactga gcatcagcaa agacaactcc aagagccaag ttttcttaaa aatgaacagt
241 ctgcaaactg atgacacagc catttactac tgtggcagag ggggtctgat tacgtccttt
301 gctatggact actggggtca aggaacctca gtcaccgtct cctcagccaa aacgacaccc
361 ccatctgtct atccactggc ccctggatct gctgcccaaa ctaactccat ggtgaccctg
421 ggatgcctgg tcaagggcta tttccctgag ccagtgacag tgacctggaa ctctggatcc
481 ctgtccagcg gtgtgcacac cttcccagct gtcctgcagt ctgacctcta cactctgagc
541 agctcagtga ctgtcccctc cagcacctgg cccagcgaga ccgtcacctg caacgttgcc
601 cacccggcca gcagcaccaa ggtggacaag aaaattgtgc ccagggattg tactagt
```

SEQ ID NO:36 (pTnMCS)

```
  1 ctgacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga
 61 ccgctacact tgccagcgcc ctagcgcccg ctcctttcgc tttcttccct tcctttctcg
121 ccacgttcgc cggcatcaga ttggttattg gccattgcat acgttgtatc catatcataa
181 tatgtacatt tatactggct catgtccaac attaccgcca tgttgacatt gattattgac
241 tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg
301 cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt
361 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca
421 atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc
481 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta
541 catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac
601 catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg
661 atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg
721 ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt
781 acggtgggag gtctatataa gcagagctcg tttagtgaac cgtcagatcc cctggagacg
```

132

```
 841 ccatccaacgc tgttttgacc tccatagaag acacogggac cgatccagcc tccgcggccg
 901 ggaacggtgc attggaacgc ggattccccg tgccaagagt gacgtaagta ccgcctatag
 961 actctatagg cacacccctt tggtcttat gcatgctata ctgtttttgg cttggggcct
1021 atacacccc gcttccttat gctataggtg atggtatagc ttagcctata ggtgtgggtt
1081 attgaccatt attgaccact ccctcattgg tgacgatact ttccattact aatccataac
1141 atggctcttt gccacaacta tctctattgg ctatatgcca atactctgtc cttcagagac
1201 tgacacggac tctgtatttt tacaggatgg ggtcccattt attatttaca aattcacata
1261 tacaacaacg ccgtcccccg tgccogcagt ttttattaaa catagcgtgg gatctccacg
1321 cgaatctcgg gtacgtgttc cggacatggg ctcttctccg gtagcggcgg agcttccaca
1381 tccggagccct ggtcccatgc ctccagccgc tcatggtcgc tcggcagctc cttgctccta
1441 acagtggagg ccagacttag gcacagcaca atgcccacca ccaccagtgt gccgcacaag
1501 gccgtggcgg tagggtatgt gtctgaaaat gagcgtggag attgggctcg cacggctgac
1561 gcagatggaa gacttaaggc agcggcagaa gaagatgcag gcagctgagt tgttgtattc
1621 tgataagagt cagaggtaac tcccgttgcg gtgctgttaa cggtggaggg cagtgtagtc
1681 tgagcagtac tcgttgctgc ggcgcgagcc accagacata atagctgaca gactaacaga
1741 ctgttccttt ccatgggtct tttctgcagt cacgtcgga ccatgtgcga actcgatatt
1801 ttacacgact ctctttacca attctgcccc gaattacact taaaacgact ccacagctta
1861 acgttggctt gccacgcatt acttgactgt aaaactctca ctcttaccga acttggccgt
1921 aacctgccaa ccaaagcgag aacaaaacat aacatcaaac gaatcgaccg attgttaggt
1981 aatcgtcacc tccacaaaga gcgactcgct gtataccgtt ggcatgctag ctttatctgt
2041 tcgggcaata cgatgccat tgtacttgtt gactggtctg atattcgtga gcaaaaacga
2101 cttatggtat tgcgagcttc agtcgcacta cacggtcgtt ctgttactct ttatgagaaa
2161 gcgttcccgc tttcagagca atgttcaaag aaagctcatg accaatttct agccgacctt
2221 gcgagcattc taccggagtaa caccacaccg ctccattgtca gtgatgctgg ctttaaagtg
2281 ccatggtata aatccgttga gaagctgggt tggtactggt taagtcgagt aagaggaaaa
2341 gtacaatatg cagacctagg agcggaaaac tggaaaccta tcagcaactt acatgatatg
2401 tcatctagtc acttcaaagac tttaggctat aagaggctga ctaaaagcaa tccaatctca
2461 tgccaaattc tattgtataa atctcgctct aaaggccgaa aaaatcagcg ctcgacacgg
2521 actcattgtc accaccogtc acctaaaatc tactcaggtc cggcaaagga gccatgggtt
2581 ctagcaacta acttacctgt tgaaattcga acacccaaac aacttgttaa tatctattcg
2641 aagcgaatgc agattgaaga aaccttccga gacttgaaaa gtcctgccta cggactaggc
2701 ctacgccata gccgaacgag cagctcagag cgttttgata tcatgctgct aatcgccctg
2761 ctgcttcaac taacatgttg gcttgcgggc gttcatgctc agaaacaagg ttgggacaag
2821 aacttccagg ctaacacagt cagaaaatgca aacgtactct caacagttcg cttaggcagg
2881 gaagtttttgc ggcattctgg ctacacaata acaaggggaag acttactcgt ggctgcaacc
2941 ctactagctc aaaatttatt cacacatggt tacgctttgg ggaaattatg aggggatcgc
3001 tctagagcga tccgggatct cgggaaaagc gttggtgacc aaaggtgcct tttatcatca
3061 ctttaaaat aaaaaacaat tactcagtgc ctgttataag cagcaattaa ttatgattga
3121 tgcctacatc acaacaaaaa ctgatttaac aaatggttgg tctgcctag aaagtatatt
3181 tgaacattat cttgattata ttattgataa taataaaaac cttatcccta tccaagaagt
3241 gatgcctatc attggttgga atgaacttga aaaaaattag ccttgaatac attactggta
3301 aggtaaacgc cattgtcagc aaattgatcc aagagaacca acttaaagct ttcctgacgg
3361 aatgttaatt ctcgttgacc ctgacgactg atgaatcccc taatgatttt ggtaaaaatc
3421 attaagttaa ggtggataca catcttgtca tatgatccca gtaatgtgag ttagctcact
3481 cattaggcac cccaggcttt acacttttatg cttccggctc gtatgttgtg tggaattgtg
3541 agcggataac aatttcacac aggaaacagc tatgaccatg attacgccaa gcgcgcaatt
3601 aaccctcact aaagggaaca aaagctggag ctccaccgcg gtggcggccg ctctagaact
3661 agtggatccc ccgggctgca ggaattcgat atcaagctta tcgataccgt cgacctcgag
3721 gggggcccg gtacccaatt cgccctatag tgagtcgtat tacgcgcgct cactggccgt
3781 cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc gccttgcagc
3841 acatccccct ttcgccagct ggcgtaatag cgaagaggcc cgcaccgatc gcccttccca
3901 acagttgcgc agcctgaatg gcgaatggaa attgtaagcg ttaatatttt gttaaaattc
3961 gcgttaaatt tttgttaaat cagctcattt ttaaccaata ggccgaaatc cggcaaaatc
4021 ccttataaat caaaagaata gacogagata gggttgagtg ttgttccagt ttggaacaag
4081 agtccactat taaagaacgt ggactccaac gtcaaagggc gaaaaaccgt ctatcagggc
4141 gatggcccac tactccggga tcatatgaca agatgtgtat ccacottaac ttaatgattt
4201 ttaccaaaat cattaggggga ttcatcagtg ctcaggtca acgagaatta acattccgtc
4261 aggaaagctt atgatgatga tgtgcttaaa aacttactca atgcatatcg
4321 caatacatgc gaaaaaccta aaagagcttg ccgataaaaa aggccaattt attgctattt
4381 accgcggctt ttattgagc ttgaaagata aataaaatag ataggtttta tttgaagcta
4441 aatcttctt atcgtaaaaa atgccctctt gggttatcaa gagggtcatt atatttcgcg
4501 aataacatc atttggttgac gaataacatt agcacttgtc tcctgtttac tccctgagc
4561 ttgaggggtt aacatgaagg tcatcgatag caggataata atacagtaaa acgctaaacc
4621 aataatccaa atccagccat cccaaattgg tagtgaatga ttataaataa cagcaaacag
4681 taatgggcca ataacaccgg ttgcattggt aaggctcacc aataatccct gtaaagcacc
4741 ttgctgatga ctctttgttt ggatagacat cactccctgt aatgcaggta aagcgatccc
4801 accaccagcc aataaaatta aaacagggaa aactaaccaa ccttcagata taaacgctaa
4861 aaaggcaaat gcactactat ctgcaataaa tccgagcagt actgccgttt tttcgcccat
```

133

```
4921 ttagtggcta ttcttcctgc cacaaaggct tggaatactg agtgtaaaag accaagaccc
4981 gtaatgaaaa gccaaccatc atgctattca tcatcacgat ttctgtaata gcaccacacc
5041 gtgctggatt ggctatcaat gcgctgaaat aataatcaac aaatggcatc gttaaataag
5101 ngatgtatac cgatcagctt ttgttccctt tagtgagggt taattgcgcg cttgggtaa
5161 tcatggtcat agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc acacaacata
5221 cgagccggaa gcataaagtg taaagcctgg ggtgcctaat gagtgagcta actcacatta
5281 attgcgttgc gctcactgcc cgctttccag tcgggaaacc tgtcgtgcca gctgcattaa
5341 tgaatcggcc aacgcgcggg gagaggcggt ttgcgtattg ggcgctcttc cgcttcctcg
5401 ctcactgact cgctgcgctc ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag
5461 gcggtaatac ggttatccac agaatcaggg gataacgcag gaaagaacat gtgagcaaaa
5521 ggccagcaaa aggccaggaa ccgtaaaaag gccgcgttgc tggcgttttt ccataggctc
5581 cgcccccctg acgagcatca caaaaatcga cgctcaagtc agaggtggcg aaacccgaca
5641 ggactataaa gataccaggc gtttccccct ggaagctccc tcgtgcgctc tcctgttccg
5701 accctgccgc ttaccggata cctgtccgcc tttctccctt cgggaagcgt ggcgctttct
5761 catagctcac gctgtaggta tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt
5821 gtgcacgaac ccccgttca gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag
5881 tccaacccgg taagacacga cttatcgcca ctggcagcag ccactggtaa caggattagc
5941 agagcgaggt atgtaggcgg tgctacagag ttcttgaagt ggtggcctaa ctacggctac
6001 actagaagga cagtatttgg tatctgcgct ctgctgaagc cagttacctt cggaaaaaga
6061 gttggtagct cttgatccgg caaacaaacc accgctggta gcggtggttt ttttgtttgc
6121 aagcagcaga ttacgcgcag aaaaaaagga tctcaagaag atcctttgat cttttctacg
6181 gggtctgacg ctcagtggaa cgaaaactca cgttaaggga ttttggtcat gagattatca
6241 aaaaggatct tcacctagat ccttttaaat taaaaatgaa gttttaaatc aatctaaagt
6301 atatatgagt aaacttggtc tgacagttac caatgcttaa tcagtgaggc acctatctca
6361 gcgatctgtc tatttcgttc atccatagtt gcctgactcc ccgtcgtgta gataactacg
6421 ntacgggagg gcttaccatc tggccccagt gctgcaatga taccgcgaga cccacgctca
6481 ccggctccag atttatcagc aataaaccag ccagccggaa gggccgagcg cagaagtggt
6541 cctgcaactt tatccgcctc catccagtct attaattgtt gccgggaagc tagagtaagt
6601 agttcgccag ttaatagttt gcgcaacgtt gttgccattg ctacaggcat cgtggtgtca
6661 cgctcgtcgt ttggtatggc ttcattcagc tccggttccc aacgatcaag gcgagttaca
6721 tgatccccca tgttgtgcaa aaaagcggtt agctccttcg gtcctccgat cgttgtcaga
6781 agtaagttgg ccgcagtgtt atcactcatg gttatggcag cactgcataa ttctcttact
6841 gtcatgccat ccgtaagatg cttttctgtg actggtgagt actcaaccaa gtcattctga
6901 gaatagtgta tgcggcgacc gagttgctct tgcccggcgt caatacggga taataccgcg
6961 ccacatagca gaactttaaa agtgctcatc attggaaaac gttcttcggg gcgaaaactc
7021 tcaaggatct taccgctgtt gagatccagt tcgatgtaac ccactcgtgc acccaactga
7081 tcttcagcat cttttacttt caccagcgtt tctgggtgag caaaaacagg aaggcaaaat
7141 gccgcaaaaa agggaataag ggcgacacgg aaatgttgaa tactcatact cttccttttt
7201 caatattatt gaagcattta tcagggttat tgtctcatga gcggatacat atttgaatgt
7261 atttagaaaa ataaacaaat aggggttccg cgcacatttc cccgaaaagt gccac
```

SEQ ID NO:37 (chicken ovalbumin ehancer)
```
ccgggctgca gaaaaatgcc aggtggacta tgaactcaca tccaaaggag
cttgacctga tacctgtgat tt tcttcaaact ggggaaacaa cacaatccca caaaacagct
cagagagaaa ccatcactga tggctacagc accaaggtat gcaatggcaa tccattcgac
attcatctgt gacctgagca aaatgattta tctctccatg aatggttgct tctttccctc
atgaaaggc aatttccaca ctcacaatat gcaacaaaga caaacagaga acaattaatg
tgctccttcc taatgtcaaa attgtagtgg caaagaggag aacaaaatct caagtctga
gtaggtttta gtgattggat aagaggcttt gacctgtgag ctcacctgga cttcatatcc
ttttggataa aaagtgcttt tataactttc aggtctccga gtctttattc atgagactgt
tggtttaggg acagacccac aatgaaatgc ctggcatagg aaagggcagc agagccttag
ctgacctttt cttgggacaa gcattgtcaa acaatgtgtg acaaaactat ttgtactgct
ttgcacagct gtgctgggca gggcaatcca ttgccaccta tcccaggtaa ccttccaact
gcaagaagat tgttgcttac tctctctaga
```

SEQ ID NO:38 (5' untranslated region)
```
GTGGATCAACATACAGCTAGAAAGCTGTATTGCCTTTAGCACTCAAGCTCAAAAGACAACTCAGAGTTC
ACC
```

SEQ ID NO:39 (putative cap site)
```
ACATACAGCTAG AAAGCTGTAT TGCCTTTAGC ACTCAAGCTC AAAAGACAAC TCAGAGTTCA
```

SEQ ID NO:40 (fragment of ovalbumin promoter - chicken)
```
GAGGTCAGAAT GGTTTCTTTA CTGTTTGTCA ATTCTATTAT TTCAATACAG
AACAATAGCT TCTATAACTG AAATATATTT GCTATTGTAT ATTATGATTG
```

134

ATLLIB02 /33492.1

```
        TCCCTCGAAC CATGAACACT CCTCCAGCTG AATTTCACAA TTCCTCTGTC
        ATCTGCCAGG CCATTAAGTT ATTCATGGAA GATCTTTGAG GAACACTGCA
        AGTTCATATC ATAAACACAT TTGAAATTGA GTATTGTTTT GCATTGTATG
        GAGCTATGTT TTGCTGTATC CTCAGAAAAA AAGTTTGTTA TAAAGCATTC
   5    ACACCCATAA AAAGATAGAT TTAAATATTC CAGCTATAGG AAAGAAAGTG
        CGTCTGCTCT TCACTCTAGT CTCAGTTGGC TCCTTCACAT GCATGCTTCT
        TTATTTCTCC TATTTTGTCA AGAAAATAAT AGGTCACGTC TTGTTCTCAC
        TTATGTCCTG CCTAGCATGG CTCAGATGCA CGTTGTAGAT ACAAGAAGGA
        TCAAATGAAA CAGACTTCTG GTCTGTTACT ACAACCATAG TAATAAGCAC
  10    ACTAACTAAT AATTGCTAAT TATGTTTTCC ATCTCTAAGG TTCCCACATT
        TTTCTGTTTT CTTAAAGATC CCATTATCTG GTTGTAACTG AAGCTCAATG
        GAACATGAGC AATATTTCCC AGTCTTCTCT CCCATCCAAC AGTCCTGATG
        GATTAGCAGA ACAGGCAGAA AACACATTGT TACCCAGAAT TAAAAACTAA
        TATTTGCTCT CCATTCAATC CAAAATGGAC CTATTGAAAC TAAAATCTAA
  15    CCCAATCCCA TTAAATGATT TCTATGGCGT CAAAGGTCAA ACTTCTGAAG
        GGAACCTGTC GCTGGGTCAC AATTCAGGCT ATATATTCCC CAGGGCTCAG
        C


        SEQ ID NO:41 pTnMCS (CMV-CHOVg-ent-ProInsulin-synPA)
  20       1 ctgacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga
          61 ccgctacact tgccagcgcc ctagcgcccg ctcctttcgc tttcttccct tcctttctcg
         121 ccacgttcgc cggcatcaga ttggcttattg gccattgcat acgttgtatc catatcataa
         181 tatgtacatt tatattggct catgtccaac attaccgcca tgttgacatt gattattgac
         241 tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg
  25     301 cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt
         361 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca
         421 atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc
         481 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta
         541 catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac
  30     601 catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg
         661 atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg
         721 ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt
         781 acggtgggag gtctatataa gcagagctcg tttagtgaac cgtcagatcg cctggagacg
         841 ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc tccgcggccg
  35     901 ggaacggtgc attggaacgc ggattccccg tgccaagagt gacgtaagta ccgcctatag
         961 actctatagg cacacccctt tggctctttat gcatgctata ctgtttttgg cttggggcct
        1021 atacacccccc gcttccttat gctataggtg atggtatagc ttagcctata ggtgtgggtt
        1081 attgaccatt attgaccact ccctattggt gacgatact ttccattact aatccataac
        1141 atggctcttt gccacaacta tctctattgg ctatatgcca atactctgtc ttcagagac
  40    1201 tgacaggac tctgtatttt tacaggatgg ggtcccattt attatttaca aattcacata
        1261 tacaacaacg ccgtcccccg tgcccgcagt ttttattaaa catagcgtgg gatcccacg
        1321 cgaatctcgg gtacgtgttc cggacatggg ctcttctccg gtagcggcgg agcttccaca
        1381 tccgagccct ggtcccatgc ctccagcggc tcatggtcgc tcggcagctc cttgctccta
        1441 acagtggagg ccagacttag gcacagcaca atgcccacca ccaccagtgt gccgcacaag
  45    1501 gccgtggcgg tagggtatgt gtctgaaaat gagcgtggag attgggctcg cacggctgac
        1561 gcagatggaa gacttaaggc agcggcagaa gaagatgcag gcagctgagt tgttgtattc
        1621 tgataagagt cagaggtaac tcccgttgcg gtgctgttaa cggtggaggg cagtgtagtc
        1681 tgagcagtac tcgttgctgc cgcgcgcgcc accagacata atagctgaca gactaacaga
        1741 ctgttccttt ccatgggtct tttctgcagt cacgtcgga ccatgtgcga actcgatatt
  50    1801 ttacacgact ctcttacca attctgcccc gaattacact taaaacgact caacagctta
        1861 acgttggctt gccacgcatt acttgactgt aaaactctca ctcttaccg acttggccgt
        1921 aacctgccaa ccaaagcgag aacaaaacat aacatcaaac gaatcgaccg attgttaggt
        1981 aatcgtcacc tccacaaaga gcgactcgct gtataccgtt ggcatgctag ctttatctgt
        2041 tcgggcaata cgatgcccat tgtacttgtt gactggtctg atattcgtga gcaaaaacga
  55    2101 cttatggtat tgcgagtctt agtcgcactaa cacggtcgtt ctgttactct ttatgagaaa
        2161 cgcgttcccgc tttcagagca atgttcaaag aaagctcatg accaattct agccgacctt
        2221 gcgagcattc taccgagtaa caccacacg ctcattgtca gtgatgctgg ctttaaagtg
        2281 ccatggtata aatccgttga gaagctgggt ggtactggt taagtcgagt aagaggaaaa
        2341 gtacaatatg cagacctagg agcggaaaac tggaaaccta tcagcaactt acatgatatg
  60    2401 tcatctagac actcaaagac tttaggctat aagaggctga ctaaaagcaa tccaatctca
        2461 tgccaaattc tattgtataa atctcgctct aaaggccgaa aaaatcagcg ctcgacacgg
        2521 tctcattgtc accacccgtc acctaaaatc tactcagcgt cggcaaagga gccatgggtt
        2581 ctagcaacta acttacctgt tgaaattcga acacccaaac aacttgttaa tatctattcg
        2641 aagcgaatgc agattgaaga aaccttccga gacttgaaaa gtcctgccta cggactaggc
  65    2701 ctacgccata gccgaacgag cagctcagag cgttttgata tcatgctgct aatcgccctg
        2761 atgcttcaac taacatgttg gcttgcgggc gttcatgctc agaaacaagg ttgggacaag
```
                                                  135

ATLUB02 133492.1

```
     2821 cacttccagg ctaacacagt cagaaatcga aacgtactct caacagttcg cttaggcatg
     2881 gaagttttgc ggcattctgg ctacacaata acaagggaag acttactcgt ggctgcaacc
     2941 ctactagctc aaaatttatt cacacatggt tacgctttgg ggaaattatg aggggatcgc
     3001 tctagagcga tccgggatct cgggaaaagc gttggtgacc aaaggtgcct tttatcatca
     3061 ctttaaaaat aaaaaacaat tactcagtgc ctgttataag cagcaattaa ttatgattga
     3121 tgcctacatc acaacaaaaa ctgatttaac aaatggttgg tctgccttag aaagtatatt
     3181 tgaacattat cttgattata ttattgataa taataaaaac cttatcccta tccaagaagt
     3241 gatgcctatc attggttgga atgaacttga aaaaaattag ccttgaatac attactggta
     3301 aggtaaacgc cattgtcagc aaattgatcc aagagaacca acttaaagct ttcctgacgg
     3361 aatgttaatt ctcgttgacc ctgagcactg atgaatcccc taatgattt ggtaaaaatc
     3421 ttaagttaa ggtggataca catcttgtca tatgatccgg gtaatgtgag ttagctcact
     3481 cattaggcac cccaggcttt acactttatg cttccggctc gtatgttgtg tggaattgtg
     3541 agcggataac aatttcacac aggaaacagc tatgaccatg attacgccaa gcgcgcaatt
     3601 aaccctcact aaagggaaca aaagctggag ctccaccgcg gtggcggccg ctctagaact
     3661 agtggatccc ccgggctatc gattggctat tggccattgc atacgttgta tccatatcat
     3721 aatatgtaca tttatattgg ctcatgtcca acattaccgc catgttgaca ttgattattg
     3781 actagttatt aatagtaatc aattacgggg tcattagttc atagcccata tatggagttc
     3841 cgcgttacat aacttacggt aaatggcccg cctggctgac cgcccaacga ccccgcca
     3901 ttgacgtcaa taatgacgta tgttcccata gtaacgccaa tagggacttt ccattgacgt
     3961 caatgggtgg agtatttacg gtaaactgcc cacttggcag tacatcaagt gtatcatatg
     4021 ccaagtacgc ccctattga cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag
     4081 tacatgacct tatgggactt tcctacttgg cagtacatct acgtattagt catcgctatt
     4141 accatggtga tgcggttttg gcagtacatc aatgggcgtg gatagcggtt tgactcacgg
     4201 ggatttccaa gtctccaccc cattgacgtc aatgggagtt tgttttggca ccaaaatcaa
     4261 cgggactttc caaaatgtcg taacaactcc gccccattga cgcaaatggg cggtaggcgt
     4321 gtacggtggg aggtctatat aagcagagct cgtttagtga accgtcagat cgcctggaga
     4381 cgccatccac gctgttttga cctccataga agacaccggg accgatccag cctccgcggc
     4441 cgggaacggt gcattggaac gcggattccc cgtgccaaga gtgacgtaag taccgcctat
     4501 agactctata ggcacacccc tttggctctt atgcatgcta tactgttttt ggcttggggc
     4561 ctatacaccc ccgcttcctt atgctatagg tgatgctaga gttagccta taggtgtggg
     4621 ttattgacca ttattgacca ctcccctatt ggtgacgata cttttccatta ctaatccata
     4681 acatggctct ttgccacaac tatctctatt ggctatatgc caatactctg tccttcagag
     4741 actgacacgg actctgtatt tttacaggat ggggtcccat ttcattattta caaattcaca
     4801 tatacaacaa cgccgtcccc cgtgcccgca gtttttatta aacatagcgt gggatctcca
     4861 cgcgaatctc gggtacgtgt tccggacatg ggctcttctc cggtaggcggc ggagcttcca
     4921 catccgagcc ctggtccat gcctccagcg gctcatggtc gctcggcagc tccttgctcc
     4981 taacagtgga ggccagactt aggcacagca caatgcccac caccaccagt gtgccgcaca
     5041 aggccgtggc ggtagggtat gtgtctgaaa atgagcgtgg agattggggct cgcacggctg
     5101 aagactagg aagcttaag gcagcgacag aagaagatgc aggcagctga gttgttgtat
     5161 tctgataaga gtcagaggta actccgttg cggtgctgtt aacggtggag ggcagtgtag
     5221 tctgagcagt actcgttgct gccgcgcgcg ccaccagaca taatagctga cagactaaca
     5281 gactgttcct ttccatgggt ccttttctgca gtcaccgtcg ggatccatgg gctccatcgg
     5341 cgcagcaagc atggaatttt gttttgatgt attcaaggag ctcaaagtcc accatgccaa
     5401 tgagaacatc ttctactgcc ccattgccat catgtcagct ctagccatg tatacctggg
     5461 tgcaaaagac agcaccagga cacagataaa taaggttgtt cgctttgata aacttccagg
     5521 attcggagac agtattgaag ctcagtgtgg cacatctgta aacgttcact cttcacttag
     5581 agacatcctc aaccaaatca ccaaccaaa tgatgtttat tcgttcagcc ttgccagtag
     5641 actttatgct gaagagagat accacaatcct gccagaatac ttgcagtgtg tgaaggaact
     5701 gtatagagga ggcttggaac ctatcaactt tcaaacagct gcagatcaag ccagagagct
     5761 catcaattcc tgggtagaaa gtcagacaaa tggaattatc agaaatgtcc ttcagccaag
     5821 ctccgtggat tctcaaactg caatggttct ggttaatgcc attgtcttca aaggactgtg
     5881 ggagaaaaca tttaaggatg aagacacaca agcaatgcct ttcagagtga ctgagcaaga
     5941 aagcaaatgt gtgcagatga tgtaccagat tggttttattt agagtggcat caatggcttc
     6001 tgagaaaatg aagatcctga gcttccatt tgccagtggg acaatgagca tgttggtgct
     6061 gttgcctgat gaagtctcag gccttgagca gcttgagagt ataatcaact ttgaaaaact
     6121 gactgaatgg accagttcta atgttatgga agagaggaag atcaaagtgt acttacctcg
     6181 catgaagatg gaggaaaat acaacctcac atctgtctta atggctatgg gcattactga
     6241 cgtgtttagc tcttcagcca atctgtctgg catctcctca gcagagacc tgaagatatc
     6301 tcaagctgtc catgcagcac atgcagaaat caatgaagca ggcagagagg tggtagggtc
     6361 agcagaggct ggagtggatg ctgcaagcgt ctctgaagaa tttagggctg accatccatt
     6421 cctcttctgt atcaagcaca tcgcaaccaa cgccgttctc ttcttggca gatgtgtttc
     6481 ccgcgccag cagatgacgc accagcagat gacgcaccag cagatgacgc accagcagat
     6541 gacgcaccag cagatgacgc accagcagat gacgcaacaa catgtatcct gaaaggctct
     6601 tgtgctgga tcggctgct ggatgacgat gacaaatttg tgaaccaaca cctgtgcggc
     6661 tcacacctgt ggaagctctc ctcctagtg tgcgggggaac gaggcttctt ctacacaccc
     6721 aagacccgcc gggaggcaga ggacctgcag gtgggcagg tggagctggg cggggggcct
     6781 ggtgcaggca gcctgcagc cttggccctg gaggggtccc tgcagaagc tggcattgtg
     6841 gaacaatgct gtaccagcat ctgctccctc taccagctgg agaactactg caactagggc
```

```
 6901 gcctaaaggg cgaattatcg cggccgctct agaccaggcg cctggatcca gatcacttct
 6961 ggctaataaa agatcagagc tctagagatc tgtgtgttgg ctttttgtgg atctgctgtg
 7021 ccttctagtt gccagccatc tgttgtttgc ccctcccccg tgccttcctt gaccctggaa
 7081 ggtgccactc ccactgtcct ttcctaataa aatgaggaaa ttgcatcgca ttgtctgagt
 7141 aggtgtcatt ctattctggg gggtggggtg gggcagcaca gcaagggggа ggattgggaa
 7201 gacaatagca ggcatgctgg ggatgcggtg ggctctatgg gtacctctct ctctctctct
 7261 ctctctctct ctctctctct ctctcggtac ctctctcgag gggggccccg gtaccaatt
 7321 cgccctatag tgagtcgtat tacgcgcgct cactggccgt cgttttacaa cgtcgtgact
 7381 gggaaaaccc tggcgttacc caacttaatc gccttgcagc acatcccccct ttcgccagct
 7441 ggcgtaatag cgaagaggcc cgcaccgatc gcccttccca acagttgcgc agcctgaatg
 7501 gcgaatggaa attgtaagcg ttaatatttt gttaaaattc gcgttaaatt tttgttaaat
 7561 cagctcattt tttaaccaat aggccgaaat cggcaaaatc ccttataaat caaaagaata
 7621 gaccgagata gggttgagtg ttgttccagt ttggaacaag agtccactat taaagaacgt
 7681 ggactccaac gtcaaagggc gaaaaaccgt ctatcagggc gatggcccac tactccggga
 7741 tcatatgaca agatgtgtat ccaccttaac ttaatgattt ttaccaaaat cattaggggа
 7801 ttcatcagtg ctcagggtca acgagaatta acattccgtc aggaaagctt atgatgatga
 7861 tgtgcttaaa aacttactca atggctgggt atgcatatcg caatacatcg gaaaaaccta
 7921 aaagagcttg ccgataaaaa aggccaattt attgctattt accgcgcgct tttattgagc
 7981 ttgaaagata aataaaatag ataggtttta tttgaagcta aatcttcttt atcgtaaaaa
 8041 atgccctctt gggttatcaa gagggtcatt atatttcgcg gaataacatc atttggtgac
 8101 gaaataaacta agcacttgtc tcctgtttac tccctgagc ttgagggtt aacatgaagg
 8161 tcatcgatag caggataata atacagtaaa acgctaaaag aataatccaa atccagccat
 8221 cccaaattgg tagtgaatga ttataaataa cagcaaacag taatgggcca ataacaccgg
 8281 ttgcattggt aaggctcacc aataatccct gtaaagcacc ttgctgatga ctctttgttt
 8341 ggatagacat cactccctgt aatgcaggta aagcgatccc accaccagcc aataaaatta
 8401 aaacagggaa aactaaccaa ccttcagata taaacgctaa aaaggcaaat gcactactat
 8461 ctgcaataaa tccgagcagt actgccgttt tttcgcccat ttagtggcta ttcttcctgc
 8521 cacaaaggct tggaatactg agtgtaaaag accaagaccc gtaatgaaaa gccaaccatc
 8581 atgctattca tcatcacgat ttctgtaata gcaccacacc gtgctggatt ggctatcaat
 8641 gcgctgaaat aataatcaac aaatggcatc gttaaataag tgatgtatac cgatcagctt
 8701 ttgttccctt tagtgagggt taattgcggc cttggcgtaa tcatggtcat agctgtttcc
 8761 tgtgtgaaat tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagtg
 8821 taaagcctgg ggtgcctaat gagtgagcta actcacatta attgcgttgc gctcactgcc
 8881 cgctttccag tcgggaaacc tgtcgtgcca gctgcattaa tgaatcggcc aacgcgcggg
 8941 gagaggcggt ttgcgtattg ggcgctcttc cgcttcctcg ctcactgact cgctgcgctc
 9001 ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag gcggtaatac ggttatccac
 9061 agaatcaggg gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa
 9121 ccgtaaaaag gccgcgttgc tggcgttttt ccataggctc cgcccccctg acgagcatca
 9181 caaaaatcga cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataccaggc
 9241 gtttccccct ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata
 9301 cctgtccgcc tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta
 9361 tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca
 9421 gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag tccaacccgg taagacacga
 9481 cttatcgcca ctggcagcag ccactggtaa caggattagc agagcgaggt atgtaggcgg
 9541 tgctacagag ttcttgaagt ggtggcctaa ctacggctac actagaagga cagtatttgg
 9601 tatctgcgct ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg
 9661 caaacaaacc accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag
 9721 aaaaaaagga tctcaagaag atcctttgat cttttctacg gggtctgacg ctcagtggaa
 9781 cgaaaactca cgttaaggga ttttggtcat gagattatca aaaaggatct tcacctagat
 9841 ccttttaaat taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc
 9901 tgacagttac caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc
 9961 atccatagtt gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc
10021 tggccccagt gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc
10081 aataaaccag ccagccggaa gggccgagcg cagaagtggt cctgcaactt tatccgcctc
10141 catccagtct attaattgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt
10201 gcgcaacgtt gttgccattg ctacaggcat cgtggtgtca cgctcgtcgt ttggtatggc
10261 ttcattcagc tccggttccc aacgatcaag gcgagttaca tgatccccca tgttgtgcaa
10321 aaaagcggtt agctccttcg gtcctccgat cgttgtcaga gtaagttggc cgcagtgtt
10381 atcactcatg gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg
10441 cttttctgtg actggtgagt actcaaccaa gtcattctga atagtgta tgcggcgacc
10501 gagttgctct tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa
10561 agtgctcatc attggaaaac gttcttcggg gcgaaaactc tcaggatct taccgctgtt
10621 gagatccagt tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt
10681 caccagcgtt tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa agggaataag
10741 ggcgacacgg aaatgttgaa tactcatact cttcctttttt caatattatt gaagcattta
10801 tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat
10861 aggggttccg cgcacatttc cccgaaaagt gccac
```

137

SEQ ID NO:42(pTnMOD (CMV-CHOVg-ent-ProInsulin-synPA))

```
   1 ctgacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga
  61 ccgctacact tgccagcgcc ctagcgcccg ctcctttcgc tttcttccct tcctttctcg
 121 ccacgttcgc cggcatcaga ttggctattg gccattgcat acgttgtatc catatcataa
 181 tatgtacatt tatattggct catgtccaac attaccgcca tgttgacatt gattattgac
 241 tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg
 301 cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt
 361 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca
 421 atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc
 481 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta
 541 catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac
 601 catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg
 661 atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg
 721 ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt
 781 acggtgggag gtctatataa gcagagctcg ttagtgaac cgtcagatcg cctggagacg
 841 ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc tccgcggccg
 901 ggaacggtgc attggaacgc ggattccccg tgccaagagt gacgtaagta ccgcctatag
 961 actctatagg cacacccctt tggctcttat gcatgctata ctgtttttgg cttggggcct
1021 atacacccc gcttccttat gctataggt atggtatagc ttagcctata ggtgtgggtt
1081 attgaccatt attgaccact ccctattgg tgacgatact ttccattact aatccataac
1141 atggctcttt gccacaacta tctctattgg ctatatgcca atactctgtc cttcagagac
1201 tgacacggac tctgtatttt tacaggatgg ggtcccattt attatttaca aattcacata
1261 tacaacaacg ccgtcccccg tgcccgcagt ttttattaaa catagcgtgg gatctccacg
1321 cgaatctcgg gtacgtgttc cggacatggg ctcttctccg gtagcggcg agcttccaca
1381 tccgacccct ggtcccatgc ctccagccgt tcatggtcgc tcggcagctc cttgctccta
1441 acagtggagg ccagacttag gcacagcaca atgcccacca ccaccagtgt gccgcacaag
1501 gccgtggcgg tagggtatgt gtctgaaaat gagcgtggag attgggctcg cacggctgac
1561 gcagatggaa gacttaaggc agcggcagaa gaagatgcag gcagctgagt tgttgtattc
1621 tgataagagt cagaggtaac tcccgttgcg gtgctgttaa cggtggaggg cagtgtagtc
1681 tgagcgatac tcgttgctgc cgcgcgatga ccagacata atagctgaca gactaacaga
1741 ctgttccttt ccatgggtct tttctgcagt caccgtcgga ccatgtgtga acttgatatt
1801 ttacatgatt ctctttacca attctgcccc gaattacact aaaaacgact caacagctta
1861 acgttggctt gccacgcatt acttgactgt aaaactctca ctcttaccga acttggccgt
1921 aacctgccaa ccaaagcgag aacaaaacat aacatccagc gaatcgaccg attgttaggt
1981 aatcgtcacc tccacaaaga gcgactccgt gtataccgtt ggcatgctag ctttatctgt
2041 tcgggcaata cgatgcccat tgtacttgtt gactggtctg atattcgtga gcaaaaacga
2101 cttatggtat tgcgagcttc agtcgcacta cacggtcgtt ctgttactct ttatgagaaa
2161 gcgttccgc tttcagagca atgttcaaag aaagctcatg accaattct agccgacctt
2221 gcgagcattc taccggatca caccacccgt ctcattgtca gtgatgtcg ctttaaagtg
2281 ccatggtata aatccgttga gaagctgggt tggtactggt taagtcgagt aagaggaaaa
2341 gtacaatatg cagacctagg agcggaaaac tggaaaccta tcagcaactt acatgatatg
2401 tcatctagtc actcaaagac tttaggctat aagaggctga ctaaaagcaa tccaatctca
2461 tgccaaattc tattgtataa atctcgctct aaaggccgaa aaaatcagcg ctcgacacgg
2521 actcattgtc accacccgtc acctaaaatc tactcagcgt cggcaaagga gccatgggtt
2581 ctagcaacta acttacctgt tgaaattcga acacccaaac aacttgttaa tatctattcg
2641 aagcgaatgc agattgaaga aaccttccga gacttgaaaa gtcctgccta cggactaggc
2701 ctacgccata gccgaacgag cagctcagag cgttttgata tcatgctgct aatcgccctg
2761 atgcttcaac taacatgttg gcttgcgggc gttcatgctc agaaacaagg ttgggacaag
2821 cacttccagg ctaacacagt cagaaatcga aacgtactct caacagttcg cttaggcatg
2881 gaagtttttgc ggcattctgg ctacacaata acaaggaag acttactcgt ggctgcaacc
2941 ctactagctc aaaatttatt cacacatggt tacgctttgg ggaaattatg ataatgatcc
3001 agatcacttc tggctaataa aagatcagag ctctagagat ctgtgtgttg gtttttttgtg
3061 ccttctagtt gccttctatttg tgcccagcca tctgttgttt gcccctccccc gtgccttcct
3121 tgaccctgga aggtgccact cccactgtcc ttcctaata aaatgaggaa attgcatcgc
3181 attgtctgag taggtgtcat tctattctgg ggggtggggt ggggcagcac agcaaggggg
3241 aggattggga agacaatagc aggcatgctg gggatgcggt gggctctatg ggtacctctc
3301 tctctctctc tctctctcta tctctcggta ccctctctct tctctctctc
3361 tctctctctc tctctctctc tcggtaccag gtgctcggta attgacccgg tgaccaaagg
3421 tgcctttat catcactttta aaataaaaa acaattactc agtgcctgtt ataagcagca
3481 attaattatg attgatgcct acatcacaac aaaaactgat ttaacaaatg gttggtctgc
3541 cttagaaagt atattgaac attatcttga ttatattatt gataataata aaaaccttat
3601 ccctatccaa gaagtgatgaa cttcattgg ttggataga cttaggaaaa attagccttg
3661 aatacattac tggtaaggta aacgccattg tcagcaaatt gatccaagag aaccaactta
3721 aagcttttct gacggaatgt taattctcgt tgaccctgag cactgatgaa tcccctaatg
3781 attttggtaa aaatcattaa gttaaggtgg atacacatct tgtcatatga tcccggtaat
3841 gtgagttagc tcactcatta ggcaccccag gctttacact ttatgcttcc ggctcgtatg
```

138

3901 ttgtgtggaa ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac
3961 gccaagcgcg caattaaccc tcactaaagg gaacaaaagc tggagctcca ccgcggtggc
4021 ggccgctcta gaactagtgg atccccgggg catcagattg gctattggcc attgcatacg
4081 ttgtatccat atcataatat gtacatttat attggctcat gtccaacatt accgccatgt
4141 tgacattgat tattgactag ttattaatag taatcaatta cggggtcatt agttcatagc
4201 ccatatatgg agttccgcgt tacataactt acggtaaatg gcccgcctgg ctgaccgccc
4261 aacgaccccc gcccattgac gtcaataatg acgtatgttc ccatagtaac gccaataggg
4321 actttccatt gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt ggcagtacat
4381 caagtgtatc atatgccaag tacgccccct attgacgtca atgacggtaa atggcccgcc
4441 tggcattatg cccagtacat gaccttatgg gactttccta cttggcagta catctacgta
4501 ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag
4561 cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt
4621 tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa
4681 atgggcggta ggcgtgtacg gtgggaggtc tatataagca gagctcgttt agtgaaccgt
4741 cagatcgcct ggagacgcca tccacgctgt tttgacctcc atagaagaca ccgggaccga
4801 tccagcctcc gcggccggga acggtgcatt ggaacgcgga ttccccgtgc caagagtgac
4861 gtaagtaccg cctatagact ctataggcac acccctttgg ctcttatgca tgctatactg
4921 tttttggctt ggggcctata cacccccgct tccttatgct ataggtgatg gtatagcttá
4981 gcctataggt gtgggttatt gaccattatt gaccactccc ctattggtga cgatactttc
5041 cattactaat ccataacatg gctctttgcc acaactatct ctattggcta tatgccaata
5101 ctctgtcctt cagagactga cacggactct gtatttttac aggatggggt cccatttatt
5161 atttacaaat tcacatatac aacaacgccg tcccccgtgc ccgcagtttt tattaaacat
5221 agcgtgggat ctccacgcga atctccgggta cgtgttccgg acatgggtc ttctccggta
5281 gcggcggagc ttccacatcc gagccctggt cccatgcctc cagcggctca tggtcgctcg
5341 gcagctcctt gctcctaaca gtggaggcca gacttaggca cagcacaatg cccaccacca
5401 ccagtgtgcc gcacaaggcc gtggcggtag ggtatgtgtc tgaaaatgag cgtggagatt
5461 gggctcgcac ggctgacgca gatgaagac ttaaggcagc ggcagaagca gatgcaggca
5521 gctgagttgt tgtattctga taagagtcag aggtaactcc cgttgcggtg gtgttaacgg
5581 tggagggcag tgtagtctga gcagtactcg ttgctgccgc gcgcgccacc agacataata
5641 gctgacagac taacagactg ttcctttcca tgggtctttt ctgcagtcac cgtcgggatc
5701 catgggctcc atcggcgcag caagcatgga attttgtttt gatgtattca aggagctcaa
5761 agtccaccat gccaatgaga acatctcota ctgccccatt gccatcatgt cagctctagc
5821 catggtatac ctgggtgcaa aagacagcac caggacacag ataaataagg ttgttcgctt
5881 tgataaactt ccaggattcg gagacagtat tgaagctcag tgtggcacat ctgtaaacgt
5941 tcactcttca cttagagaca tcctcaacca aatcaccaaa ccaaatgatg tttattcgtt
6001 cagccttgcc agtagacttt atgctgaaga gagataccca atcctgccaa aatacttgca
6061 gtgtgtgaag gaactgtata gaggaggctt ggaacctatc aacttcaaa cagctgcaga
6121 tcaagccaga gagctcatca attcctgggt agaaagtcag acaaatggaa ttatcagaaa
6181 tgtccttcag ccaagctccg tggattctca aactgcaatg gttctggtta atgccattgt
6241 cttcaaagga ctgtgggaga aacatttaa ggatgaagac acacaagcaa tgccttcag
6301 agtgactgag caagaaagca acctgtgca gatgatgtac cagattggtt tatttagagt
6361 ggcatcaatg gcttctgaga aaatgaagat cctggagctt ccatttggca gtgggacaat
6421 gagcatgttg gtgctgttgc ctgatgaagt ctcaggcctt gagcagcttg agagtataat
6481 caactttgaa aaactgactg aatggaccag ttctaatgtt atggaagaga ggaagatcaa
6541 agtgtactta cctcgcatga agatggagga aaaatacaac ctcacatctg tcttaatggc
6601 tatgggcatt actgacgtgt ttagctcttc agccaatctg tctggcatct cctcagcaga
6661 gagcctgaag atatctcaag ctgtccatgc agcacatgca gaaatcaatg aagcaggcag
6721 agaggtggta gggtcagcag aggctggagt ggatgctgca agcgtctctg aagaatttag
6781 ggctgaccat ccattcctct tctgtatcaa gcacatcgca accaacgccg ttctctctt
6841 tggcagatgt gtttcccgcg gccagcagat gacgcaccag cagatgacgc accagcagat
6901 gacgcaccag cagatgacgc accagcagat gacgcaccag cagatgacga aacaacatgt
6961 atcctgaaag gctcttgtgg ctggatcggc ctgctgatcg acgatgacaa atttgtgaac
7021 caacacctgt gcggctcaca cctggtggaa gctctctacc tagtgtgcgg ggaacgaggc
7081 ttcttctaca cacccaagac ccgccgggag gcagaggacc tgcagtgggg gcaggtggag
7141 ctgggcgggg gccctgttgc ggcagcgctg cagccctgcc cctgagggg gtccctgcag
7201 aagcgtggca ttgtggaaca atgctgtacc agcatctgct ccctctacca gctggagaac
7261 tactgcaact agggcgccta aagggcgaat tatcgcggcc gctctagaca aggcgcctgg
7321 atccagatca cttctggcta ataaagatc agagctctag agatctgtgt gttggttttt
7381 tgtggatctg ctgtgccttc tagttgccag ccatctgttg tttgccctc cccgtgcct
7441 tccttgaccc tggaaggtgc cactcccact gtcctttcct aataaaatga ggaaattgca
7501 tcgcattgtc tgagtaggtg tcattctatt ctggggggtg gggtggggca gcacagcaag
7561 ggggaggatt gggaagacaa tagcaggcat gctggggatg cggtgggctc tatgggtacc
7621 tctctctctc tctctctc tctctctctc tctctctctc ggtacctctc ctcgagggg
7681 ggccccggtac ccaattcgcc ctatagtgag tcgtattacg cgcgctcact ggccgtcgtt
7741 ttacaacgtc gtgactggga aaaccctggc gttacccaac ttaatcgcct tgcagcacat
7801 cccccttttcg ccagctggcg taatagcgaa gaggcccgca ccgatcgccc ttcccaacag
7861 ttgcgcagcc tgaatggcga atggaaattg taagcgttaa tattttgtta aaattcgcgt
7921 taaatttttg ttaaatcagc tcattttttta accaataggc cgaaatcggc aaaatccctt

139

```
        7981 ataaatcaaa agaatagacc ggagataggg  tgagtgttgt tccagtttgg aacaagagtc
        3041 cactattaaa gaacgtggac tccaacgtca aagggcgaaa aaccgtctat cagggcgatg
        8101 gcccactact ccgggatcat atgacaagat gtgtatccac cttaacttaa tgattttac
        8161 caaaatcatt aggggattca tcagtgctca gggtcaacga gaatcaacat tccgtcagga
        8221 aagcttatga tgatgatgtg cttaaaaact tactcaatgg ctggttatgc atatcgcaat
        4281 acatgcgaaa aacctaaaag agcttgccga taaaaaaggc caatttattg ctatttaccg
        8341 cggcttttta ttgagcttga aagataaata aaatagatat gttttatttg aagctaaatc
        8401 ttctttatcg taaaaaatgc cctcttgggt tatcaagagg gtcattatat ttcgcggaat
        8461 aacatcattt ggtgacgaaa taactaagca cttgtctcct gtttactccc ctgagcttga
        8521 ggggttaaca tgaaggtcat cgatagcagg ataataatac agtaaaacgc taaaccaata
        8581 atccaaatcc agccatccca aattggtagt gaatgattat aaataacagc aaacagtaat
        8641 gggccaatca cacccggttgc attggtaagg ctcaccaata atccctgtaa agcaccttgc
        8701 tgatgactct ttgtttggat agacatcact ccctgtaatg caggtaaagc gatcccacca
        8761 ccagccaata aaattaaaac agggaaaact aaccaacctt cagatataaa cgctaaaaag
        8821 gcaaatgcac tactatctgc aataaatccg agcagtactg ccgttttttc gcccatttag
        8881 tggctattct tcctgccaca aaggcttgga atactgagtg taaaagacca agacccgtaa
        8941 tgaaaagcca accatcatgc tattcatcat cacgatttct gtaatagcac cacaccgtgc
        9001 tggattggct atcaatgcgc tgaaataata atcaacaaat ggcatcgtta aataagtgat
        9061 gtatacgat cagctttgt tccctttagt gagggttaat tgcgcgcttg ggtaatcat
        9121 ggtcatagct gtttcctgtg tgaaattgtt atccgctcac aattccacac aacatacgag
        9181 ccggaagcat aaagtgtaaa gcctggggtg cctaatgagt gagctaactc acattaattg
        9241 cgttgcgctc actgcccgct ttccagtcgg gaaacctgtc gtgccagctg cattaatgaa
        9301 tcggccaacg cgcggggaga ggcggtttgc gtattgggcg ctcttccgct tcctcgctca
        9361 ctgactcgct gcgctcggtc gttcggctgc ggcgagcggt atcagctcac tcaaaggcgg
        9421 taatacggtt atccacagaa tcaggggata gacaggaaa gaacatgtga gcaaaaggcc
        9481 agcaaaaggc caggaaccgt aaaaaggccg cgttgctggc gtttttccat aggctccgcc
        9541 ccctgacga gcatcacaaa aatcgacgct caagtcagag gtggcgaaac ccgacaggac
        9601 tataaagata ccaggcgttt ccccctggaa gctccctcgt gcgctctcct gttccgaccc
        9661 tgccgcttac cggatacctg tccgcctttc tcccttcggg aagcgtggcg ctttctcata
        9721 gctcacgctg taggtatctc agttcggtg aggtcgttcg ctccaagctg ggctgtgtgc
        9781 acgaacccc cgttcagccc gaccgctgcg ccttatccgg taactatcgt cttgagtcca
        9841 acccggtaag acacgactta tcgccactgg cagcagccac tggtaacagg attagcagag
        9901 cgaggtatgt aggcggtgct acagagttct tgaagtggtg gcctaactac ggctacacta
        9961 gaaggacagt atttggtatc tgcgctctgc tgaagccagt taccttcgga aaaagagttg
       10021 gtagctcttg atccggcaaa caaaccaccg ctggtagcgg tggtttttt gtttgcaagc
       10081 agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt tctacggggt
       10141 ctgacgctca gtggaacgaa aactcacgtt aagggatttt ggtcatgaga ttatcaaaaa
       10201 ggatcttcac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc taaagtatat
       10261 atgagtaaac ttggtctgac agttaccaat gcttaatcag tgaggcacct atctcagcga
       10321 tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata actacgatac
       10381 gggagggctt accatctggc cccagtgctg caatgatacc gcgagaccca cgctcaccgg
       10441 ctccagattt atcagcaata aaccagccag ccggaagggc cgagcgcaga agtggtcctg
       10501 caactttatc cgcctccatc cagtctatta ttgttgccg ggaagctaga gtaagtagtt
       10561 cgccagttaa tagtttgcgc aacgttgttg ccattgctac aggcatcgtg gtgtcaacgct
       10621 cgtcgtttgg tatggcttca ttcagctccg gttcccaacg atcaaggcga gttacatgat
       10681 cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc tccgatcgtt gtcagaagta
       10741 agttggccgc agtgttatca ctcatggtta tggcagcact gcataattct cttactgtca
       10801 tgccatccgt aagatgcttt tctgtgactg gtgagtactc aaccaagtca ttctgagaat
       10861 agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat acggataat accgcgccac
       10921 atagcagaac tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga aaactctcaa
       10981 ggatcttacc gctgttgaga tccagttcga tgtaacccac tcgtgcaccc aactgatctt
       11041 cagcatcttt tactttcacc agcgtttctg ggtgagcaaa aacaggaagg caaaatgccg
       11101 caaaaaaggg aataagggcg acacggaaat gttgaatact catactcttc ctttttcaat
       11161 attattgaag catttatcag ggttattgtc tcatgagcgg atacatattt gaatgtattt
       11221 agaaaaataa acaaataggg gttccgcgca catttccccg aaaagtgcca c
```

SEQ ID NO:43 (pTnMOD(Chicken OVep+OVg'+ENT+proins+syn polyA))

```
          1 ctgacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga
         61 ccgctacact tgccagcgcc ctagcgcccg ctcctttcgc tttcttccct tccttctcg
        121 ccacgttcgc cggcatcaga ttggctattg gccattgcat acgttgtatc catatcataa
        181 tatgtacatt tatattggct catgtccaac attaccgcca tgttgacatt gattattgac
       -241 tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg
       -301 cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc ccgcccatt
       -361 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca
```

140

```
 421 atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc
 481 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta
 541 catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac
 601 catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg
 661 atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg
 721 ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt
 781 acggtgggag gtctatataa gcagagctcg tttagtgaac cgtcagatcg cctggagacg
 841 ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc tccgcggccg
 901 ggaacggtgc attggaacgc ggattccccg tgccaagagt gacgtaagta ccgcctatag
 961 actctatagg cacaccccta tggctcttat gcatgctata ctgttttggg cttggggcct
1021 atacacccc gcttccttat gctataggtg atggtatagc ttagcctata ggtgtgggtt
1081 attgaccatt attgaccact ccctattgg tgacgatact ttccattact aatccataac
1141 atggctcttt gccacaacta tctctattgg ctatatgcca atactctgtc cttcagagac
1201 tgacacggac tctgtatttt tacaggatgg ggtcccattt attatttaca aattcacata
1261 tacaacaacg ccgtcaccac tttttattaaa catagacgcgg gatctccacg
1321 cgaatctcgg gtacgtgttc cggacatggc ctcttctccg gtagcggcgg agcttccaca
1381 tccgagccct ggtcccatgc ctccagcggc tcatggtcgc tcggcagctc cttgctccta
1441 acagtggagg ccagacttag gcacagcaca atgcccacca ccaccagtgt gccgcacaag
1501 gccgtggcgg tagggtatgt gtctgaaaat gagcgtggag attgggctcg cacggctgac
1561 gcagatggaa gacttaaggc agcggcagaa gaagatgcag gcagctgagt tgttgtattc
1621 tgataagagt cagaggtaac tcccgttgcg gtgctgttaa cggtggaggg cagtgtagtc
1681 tgagcagtac tcgttgctgc cgcgcgcgcc accagacata atagctgaca gactaacaga
1741 ctgttccttt ccatgggtct tttctgcagt caccgtcgga ccatgtgcga actcgatatt
1801 ttacacgact ctctttacca attctgcccc gaattacact taaaacgact caacagctta
1861 acgttggctt gccacgcatt acttgactgt aaaactctca ctcttaccga acttggccgt
1921 aacctgccaa ccaaagcgag aacaaaacat aacatcaaac gaatcgaccg attgttaggt
1981 aatcgtcacc tccacaaaga gcgactcgct gtataccgtt ggcatgctag ctttatctgt
2041 tcgggcaata cgatgcccat tgtacttgct gactggtctg atattcgtga gcaaaaacga
2101 cttatggtat tgcgagcttc agtcgtacta cacggtcgtt ctgttactct ttatgagaaa
2161 gcgttcccgc tttcagagca atgttcaaag aaagctcatg accaatttct agccgacctt
2221 gcgagcattc tacgagtaa caccacaccg ctcattgtca gtgatgctgg ctttaaagtg
2281 ccatggtata aatccgttga gaagctgggt tggtactggt taagtcgagt aagaggaaaa
2341 gtacaatatg cagacctagg agcggaaaac tggaaaccta tcagcaactt acatgatatg
2401 tcatctagtc actaaagagc tttaggctat aagaggctga ctaaaagcaa tccaatctca
2461 tgccaaattc tattgtataa atctcgctct aaaggccgaa aaaatcagcg ctcgacacgg
2521 actcattgtc accacccgtc acctaaaatc tactcagcgt cggcaaagga gccatggtt
2581 ctagcaacta acttacctgt tgaaattcga acaccaaac aacttgttaa tatctattcg
2641 aagcgaatgc agattgaaga aaccttccga gacttgaaaa gtcctgccta cggactaggc
2701 ctacgccata gccgaacgag cgtttttgata tcatgctgct aatcgccctg
2761 atgcttcaac taacatgttg gcttgggggc gttcatgctc agaaacaagg ttgggacaag
2821 cacttccagg ctaacacagt cagaaatcga aacgtactct caacagttcg cttaggcatg
2881 gaagtttgc ggcattctgg ctacacaata acaagggaag acttactcgt ggctgcaacc
2941 ctactagctc aaaatttatt cacacatggt tacgctttgg ggaattatg aggggatcgc
3001 tctagagcga tccgggatcc cgggaaaagc gttggtgacc aaaggtgcct tttatcatca
3061 ctttaaaaat aaaaaacaat tactcagtgc ctgttataag cagcaattaa ttatgattga
3121 tgcctacatc acaacaaaaa ctgatttaac aaatggttgg tctgcttag aaagtatatt
3181 tgaacattat cttgattata ttattgataa taataaaaac cttatcccta tccaagaagt
3241 gatgcctatc attggttgga atgaacttga aaaaaattag ccttgaatac attactggta
3301 aggtaaacgc cattgtcagc aaattgatcc aagagaacca acttaaagct ttcctgacgg
3361 aatgttaatt ctcgttgacc ctgagcactg atgaatcccc taatgattt ggtaaaaatc
3421 attaagttaa ggtggataca catcttgtca tatgatcccg gtaatgtgag ttagctcact
3481 cattaggcac cccaggcttt acactttatg cttccggctc gtatgttgtg tggaattgtg
3541 agcggataac aatttcacac aggaaacagc tatgaccatg attacgccaa gcgcgcaatt
3601 aaccctcact aaagggaaca aagctggagc tccaccgcgg tggcggccg ctctagaact
3661 agtggatccc ccgggctgca gaaaatgcc aggtggacta tgaactcaca tccaaaggag
3721 cttgacctga tacctgattt tcttcaaact ggggaacaa cacaatccca caaaacagct
3781 cagagagaaa ccatcactga tggctacaac accaaggtat gcaatggcaa tccattcgac
3841 attcatctgt gacctgagca aaatgattta tctctccatg aatggttgct tctttccctc
3901 atgaaaggc aatttccaca ctcacaatat gcaacaaaga caaacagaga acaattaatg
3961 tgctccttcc taatgtcaaa attgtagtgg caaagaggag aacaaaatct caagttctga
4021 gtaggtttta gtgattggat aagaggcttt gacctgtgag ctcacctgga cttcatatcc
4081 ttttggataa aaagtgcttt tataacttttc aggtcctcga gtctttattc atgagactgt
4141 tggtttaggg acagacccac aatgaaatgc ctggcatagg aaagggcagc agagccttag
4201 ctgaccttt cttgggacaa gcattgtcaa acaatgtgtg acaaactat ttgtactgct
4261 ttgcacagct gtgctgggca gggcaatcca ttgccaccta tccaggtaa ccttccaact
4321 gcaagaagat tgttgcttac tctctctaga aagcttctgc agactgacat gcattcata
4381 ggtagagata acattactg ggaagcacat ctatcatcat aaaaagcagg caagatttc
4441 agacttcctt agtggctgaa atagaagcaa aagacgtgat taaaacaaa atgaaacaaa
```

141

```
       4501 aaaaatcagt tgatacctgt ggtgtagaca tccagcaaaa aaatattatt tgcactacca
       4561 tcttgtctta agtcctcaga cttggcaagg agaatgtaga tttctacagt atatatgttt
       4621 tcacaaaagg aaggagagaa acaaaagaaa atggcactga ctaaacttca gctagtggta
       4681 taggaaagta attctgctta acagagattg cagtgatctc tatgtatgtc ctgaagaatt
  5    4741 atgttgtact ttttttcccc attttttaaat caaacagtgc tttacagagg tcagaatggt
       4801 ttctttactg tttgtcaatt ctattattgtc aatacagaac aatagcttct ataactgaaa
       4861 tatatttgct attgtatatt atgattgtcc ctcgaaccat gaacactcct ccagctgaat
       4921 ttcacaattc ctctgtcatc tgccaggcca ttaagttatt catggaagat ctttgaggaa
       4981 cactgcaagt tcatatcata aacacatttg aaattgagta ttgtttttgca ttgtatggag
  10   5041 ctatgttttg ctgtatcctc agaaaaaaag tttgttataa agcattcaca cccataaaaa
       5101 gatagattta aatattccag ctataggaaa gaaagtgcgt ctgctcttca ctctagtctc
       5161 agttggctcc ttcacatgca tgcttcttta tttctcctat tttgtcaaga aaataatagg
       5221 tcacgtcttg ttctcactta tgtcctgcct agcatggctc agatgcacgt tgtagataca
       5281 agaaggatca aatgaaacag acttctggtc tgttactaca accatagtaa taagcacact
  15   5341 aactaataat tgctaattat gttttccatc tctaaggttc ccacattttt ctgtttctt
       5401 aaagatccca ttatctggtt gtaactgaag ctcaatggaa catgagcaat attttcccagt
       5461 cttctctccc atccaacagt cctgatggat tagcagaaca ggcagaaaac acattgttac
       5521 ccagaattaa aaactaatat ttgctctcca ttcaatccaa aatggaccta ttgaaactaa
       5581 aatctaaccc aatcccatta aatgattttct atggcgtcaa aggtcaaact tctgaaggga
  20   5641 acctgtgggt gggtcacaat tcaggctata tattccccag ggctcagcca gtggatcaac
       5701 atacagctag aaagctgtat tgcccttagc actcaagctc aaaagacaac tcagagttca
       5761 ccatgggctc catcggcgca gcaagcatgg aattttgttt tgatgtattc aaggagctca
       5821 aagtccacca tgccaatgag aacatcttct actgcccat tgccatcatg tcagctctag
  25   5881 ccatggtata cctgggtgca aaagacagca ccaggacaca gataaataag gttgttcgct
       5941 ttgataaact tccaggattc ggagacagta ttgaagctca gtgtggcaca tctgtaaacg
       6001 ttcactcttc acttagagac ttcctcaacc aaatcaccaa accaaatgat gtttattcgt
       6061 tcagccttgc cagtagactt tatgctgaag agagataccc aatcctgcca gaatacttgc
       6121 agtgtgtgaa ggaactgtat agaggaggct tggaacctat caactttcaa acagctgcag
  30   6181 atcaagccag agagctcatc aattcctggg tagaaagtca gacaaatgga attatcagaa
       6241 atgtccttca gccaagctcc gtggattctc aaactgcaat ggttctggtt aatgccattg
       6301 tcttcaaagg actgtgggag aaaacattta aggatgaaga cacacaagca atgcctttca
       6361 gagtgactga gcaagaaagc aaacctgtgc agatgatgta ccagattggt ttatttagag
       6421 tggcatcaat ggcttctgag aaaatgaaga tcctggagct tccatttgcc agtgggacaa
  35   6481 tgagcatgtt ggtgctgttg cctgatgaag tctcaggcct tgagcagctt gagagtataa
       6541 tcaactttga aaaactgact gaatggacca gttctaatgt tatggaagag aggaagatca
       6601 aagtgtactt acctcgcatg aagatggagg aaaaatacaa cctcacatct gtcttaatgg
       6661 ctatgggcat tactgacgtg tttagctctt cagccaatct gtctggcatc tcctcagcag
       6721 agagcctgaa gatatctcaa gctgtccatg cagcacatgc agaaatcaat gaagcaggca
  40   6781 gagaggtggt agggtcagca gaggctggag tggatgctgc aagcgtctct gaagaattta
       6841 gcttcttcta cacacccaag acccgcagg aggcaggaga cctgcaggtg gggcaggtgg
       6901 ttggcagatg tgttctccg cggccagcac atgacgcacc agcagatgac gcaccagcag
       6961 atgacgcacc agcagatgac gcaccagcag atgacgcacc agcagatgac gcaacaacat
       7021 gtatcctgaa aggctcttgt ggctggatcg gcctgctgga tgacgatgac aaatttgtga
  45   7081 accaacacct gtgcggctca cacctggtgg aagctctcta cctagtgtgc ggggaacgag
       7141 gcttcttcta cacacccaag accctgcagg aggcaggaga cctgcaggtg gggcaggtgg
       7201 agctgggcgg gggccctggt gcaggcagca tgcagccctt ggccctggag gggtccctgc
       7261 agaagcgtgg cattgtggaa caatgctgta ccagcatctg ctccctctac cagctggaga
       7321 actactgcaa ctagggcgcc taaagggcga attatcgcgg ccgctctaga ccaggcgcct
  50   7381 ggatccagat cacttctggc taataaaaga tcagagctct agagatctgt gtgttggttt
       7441 ttgtggatgc tgctgtgcct tctagttgcc agccatctgt tgtttgcccc tccccgtgc
       7501 cttccttgac cctggaaggt gccactccca ctgtcctttc ctaataaaat gaggaaattg
       7561 catcgcattg tctgagtagg tgtcattcta ttctggggg tggggtgggg cagcacagca
       7621 agggggagga ttgggaagac aatagcaggc atgctgggga tgcggtgggc tctatgggta
  55   7681 gggcctctctc tctctctctc tctctctctc tctctctctc tcggtacctc tctcggatgg
       7741 gggcccggta cccaattcgc cctatagtgt gtcgtattac gcgcgctcac tggccgtcgt
       7801 tttacaacgt cgtgactggg aaaaccctgg cgttacccaa cttaatcgcc ttgcagcaca
       7861 tccccctttc gccagctggc gtaatagcga agaggcccgc accgatcgcc cttcccaaca
       7921 gttgcgcagc ctgaatggcg aatggaaatt gtaagcgtta tatttttgtt aaaattcgcg
  60   7981 ttaaatttttt gttaaatcagg ctcattttat aaccaatagg ccgaaatcgg caaaatcccct
       8041 tataaatcaa aagaatagac cgagataggg ttgagtgttg ttccagtttg gaacaagagt
       8101 ccactattaa agaacgtgga ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat
       8161 ggcccactac tccgggatca tatgacaaga tgtgtatcca ccttaactta atgattttta
       8221 acaaaatcat taggggattc atcagtgctc agggtaacg agaattaaca ttccgtcagg
  65   8281 aaagcttatg atgatgatgt gcttcacat ttactcaatg gctggttatg catatcgcaa
       8341 tacatgcgaa aaacctaaaa gagcttgcg ataaaaaagg ccaatttatt gctatttacc
       8401 gcggcttttt attgagcttg aaagataaat aaaatagata ggtttttattt gaagctaaat
       8461 cttctttatc gtaaaaaatg ccctcttggg ttatcaagag ggtcattata tttcgcggaa
       8521 taacatcatt tggtgacgaa ataactaagc acttgtctcc tgtttactcc cctgagcttg
```

                                           142

```
     8581 aggggttaac atgaaggtca tcgatagcag gataataata cagtaaaacg ctaaaccaat
     8641 aatccaaatc cagccatccc aaattggtag tgaatgatta taaataacag caaacagtaa
     8701 tgggccaata acaccggttg cattggtaag gctcaccaat aatccctgta aagcaccttg
     8761 ctgatgactc tttgtttgga tagacatcac tccctgtaat gcaggtaaag cgatcccacc
5    8821 accagccaat aaaattaaaa cagggaaaac taaccaacct tcagatataa acgctaaaaa
     8881 ggcaaatgca ctactatctg caataaatcc gagcagtact gccgtttttt cgcccattta
     8941 gtggctattc ttcctgccac aaaggcttgg aatactgagt gtaaaagacc aagacccgta
     9001 atgaaaagcc aaccatcatg ctattcatca tcacgatttc tgtaatagca ccacaccgtg
     9061 ctggattggc tatcaatgcg ctgaaataat aatcaacaaa tggcatcgtt aaataagtga
10   9121 tgtataccga tcagcttttg ttccctttag tgagggttaa ttgcgcgctt ggcgtaatca
     9181 tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga
     9241 gccggaagca taaagtgtaa agcctggggt gcctaatgag tgagctaact cacattaatt
     9301 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga
     9361 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc
15   9421 actgactcgc tgcgctcggt cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg
     9481 gtaatacggt tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc
     9541 cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc
     9601 ccccctgacg agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga
     9661 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc
20   9721 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat
     9781 agctcacgct gtaggtatct cagttcggtg taggtcgttc gctccaagct gggctgtgtg
     9841 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc
     9901 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacta gattagcaga
     9961 gcgaggtatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact
25   10021 agaaggacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt
     10081 ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag
     10141 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg
     10201 tctgacgctc agtggaacga aaactcacgt taagggattt tggtcatgag attatcaaaa
     10261 aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata
30   10321 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg
     10381 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata
     10441 cgggagggct taccatctgg ccccagtgct gcaatgatac cgcgagaccc acgctcaccg
     10501 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag aagtggtcct
     10561 gcaacttta t ccgcctccat ccagtctatt aattgttgcc gggaagctag agtaagtagt
35   10621 tcgccagtta atagtttgcg caacgttgtt gccattgcta caggcatcgt ggtgtcacgc
     10681 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga
     10741 tcccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt
     10801 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc
     10861 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa
40   10921 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca
     10981 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca
     11041 aggatcttac cgctgttgag atccagttcg atgtaaccca ctcgtgcacc caactgatct
     11101 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc
     11161 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt cctttttcaa
45   11221 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt
     11281 tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc ac
```